Uterus autotransplantation in cynomolgus macaques: intraoperative evaluation of uterine blood flow using indocyanine green

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BACKGROUND: Uterus transplantation may be the only theoretical option for some women, for example, those with congenital uterine infertility or who have undergone hysterectomy. In this study, we evaluated the intra- and post-operative blood flow conditions of vascular anastomosed regions and the blood-perfused area of the transplanted uterus in a cynomolgus macaque model of uterus autotransplantation.

METHODS: Female cynomolgus monkeys (n = 6) underwent surgery: the first two animals were used to study the pelvic vascular anatomy and the remaining four animals were used for uterus autotransplantation. We used indocyanine green (ICG) fluorescent angiography during surgery to assess blood perfusion in the vascular anastomosed region and uterine area. After surgery, the uterine size, presence or absence of the endometrium and blood flow rates in the uterine artery and vein were evaluated using Doppler ultrasonography.

RESULTS: Uterine arterial and venous anastomoses succeeded in all four animals that underwent autotransplantation. Intraoperative ICG fluorescence angiography showed favorable blood flow in the vascular anastomosed region and the entire uterus received a sufficient blood supply from a single uterine artery. Favorable blood flow in the uterine artery and vein immediately after surgery was shown by Doppler ultrasonography. Ultimately, three out of four animals died within 3 months following surgery because of reduced feeding and loss of body strength.

CONCLUSIONS: ICG fluorescence angiography can be used for simple evaluation of real-time blood flow conditions in the anastomosed uterine artery, vein and uterine area and can facilitate the success rate of uterus transplantation.

Key words: cynomolgus monkey / uterus transplantation / microsurgery / uterine blood flow / autotransplantation

Introduction

Women requiring hysterectomy for a malignant uterine tumor or benign disease or those with massive bleeding after delivery or congenital uterine infertility associated with defects of the uterus and vagina (Rokitansky syndrome) cannot bear subsequent children, which can be a major obstacle to love and marriage and affects the woman’s quality of life (Beski et al., 2000; Grynb erg et al., 2011). Uterus transplantation may be an option for these patients, but experiments in this area have been limited to small laboratory animals, such as mice (Racho El-Akouri et al., 2003a,b,2006) and rats (Salehian et al., 2008; Diaz-Garcia et al., 2010; Wranning et al., 2011), and large experimental animals, such as pigs (Avison et al., 2009), sheep (Ramirez et al., 2011) and non-human primates (Enskog et al., 2010; Kisu et al., 2011).

Maintenance of uterine blood flow is important for uterus transplantation and subsequent maintenance of pregnancy. The world’s first human uterus transplantation was performed in Saudi Arabia in...
2000, but the uterus necrotized because of a vascular problem 99 days after surgery, leading to transplantation failure in this case (Fageeh et al., 2002). The blood vessels that feed the uterus include the bilateral uterine arteries and veins, deep uterine veins and ovarian arteries and veins. Selection of blood vessels for uterus transplantation determines the difficulty of the surgery and is closely associated with subsequent maintenance of pregnancy. However, few reports have described the perfused area of each blood vessel. Although it is proposed that gestation and delivery are possible with only the ovarian artery and vein, it is also known that decreased ovarian function occurs without the uterine artery and vein (Cordonnier et al., 2002; Pinto Pabon et al., 2008; Gaia et al., 2009). Regarding clinical applications, ovaries will not be transplanted and there is a possibility that the ovarian artery and vein cannot be used for anastomosis. Therefore, it is important to establish a procedure in which the uterine artery and vein are used. Echography is used to monitor post-operative blood flow in various organ transplantations, but an intraoperative method to investigate blood flow in the anastomosed regions or perfused areas has not been established. Therefore, the objective of this study was to evaluate intra- and post-operative blood flow in the vascular anastomosed regions and the perfused area in the transplanted uterus in an animal model using indocyanine green (ICG) fluorescence angiography and Doppler ultrasonography.

**Materials and Methods**

**Animals**

The study protocol was approved by the institutional scientific evaluation and review committee and the animal care and use committee of the Institute of Primate Research, Shin-Nihon-Kagaku, Kagoshima, Japan. Six healthy adult female cynomolgus monkeys (2.85–4.3 kg) with regular menstrual cycles for several months before the experiments were used in this study, including two monkeys which we reported previously (Kisu et al., 2011). The animals were fed a commercial monkey diet once daily, with supplemental fruits and vegetables seven times weekly. A dissection study of the uterine artery and vein, ovarian artery and vein and deep uterine vein feeding the uterus was performed in two of the six animals (Fig. 1a and b) and then four animals underwent autotransplantation of the uterus.

**Anesthesia**

After sedation with atropine sulfate (0.02 ml/kg, i.m. injection; Mitsubishi Tanabe Pharma Corp, Tokyo, Japan) and ketamine hydrochloride (50 mg/ml aqueous solution, 0.1–0.2 ml/kg i.m.; Kamud Drugs, Tokyo, Japan), the abdomen was shaved and the animal was maintained in the supine position. The abdomen was disinfected with 70% ethanol and iodine tincture and covered with a sterilized drape. A tracheal tube was inserted and anesthesia was maintained with isoflurane inhalation (0.5–1.0% inhalation; Abbott Japan, Tokyo, Japan). During autotransplantation, anesthesia was maintained with inhalation of a mixture of carrier gas (nitrous oxide/oxygen, 2:1) and 1% isoflurane using an inhalant anesthesia vaporizer for isoflurane (AIV-5; Kimura Medical Co., Tokyo, Japan) and equipment for general anesthesia (Kimura General Anesthesia Device Compact-15, Kimura Medical Co.) under spontaneous respiration. To compensate for fluid loss during surgery, the animal received continuous i.v. infusion of 0.163 M NaCl at 3–4 ml/kg/h, according to our protocol for administering perioperative fluid during pediatric surgeries at the University of Tokyo Hospital and University of Keio Hospital. Heparin was not given to prevent intraoperative bleeding, based on our experience from free jejunum autotransplantation for esophageal cancer in humans.

After extubation, the animal was kept in a single cage to allow administration of medication and for further observation. Antibiotics (dihydrostreptomycin sulfate 0.02–0.1 ml/kg i.m.; Meiji Seika, Tokyo, Japan) and buprenorphine hydrochloride (Lepetan 0.005–0.001 mg/kg i.m.; Otsuka Pharmaceutical Co., Tokyo, Japan) were administered for 3 days after autotransplantation to prevent infection and to treat pain, respectively. Lactated Ringer’s solution was administered i.v. if there was a change in the post-transplantation condition, such as appetite loss or dehydration. The animal was not given heparin to prevent post-operative bleeding. We performed second-look laparoscopy 2–4 months after surgery and laparotomy 4–19 months after primary surgery using identical methods for induction and maintenance of anesthesia as described for primary surgery. The animals were euthanized by i.p. administration of an aqueous solution of pentobarbital sodium (64.8 mg/ml, 4.0 ml/kg) at the completion of the study.

**Figure 1** Uterine anatomy in cynomolgus macaque monkeys. (a) Frontal view while processing the left broad ligament of the uterus. (b) Right lateral view of the uterus. The yellow arrow indicates the right uterine artery and vein.
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Retrieval surgery

The cynomolgus monkey was positioned in a dorsiventr al recumbent position and the abdomen was shaped, disinfected with 1% chlorhexidine solution, and draped. The vagina was cleaned with 0.1% chlorhexidine solution and the urinary bladder was emptied via a transcutaneous bladder drainage catheter, which was left in place during surgery to enable measurement of urinary output. All surgical procedures were performed using sterile techniques and surgical loupes (magnification × 2.3, × 5.5). A midline incision was made from the pubic bone to the level of the umbilicus and the intestines were packed into the upper abdomen. The vascular anatomy of the internal genital organs of the cynomolgus monkey resembles that of the human female (Fig. 1a and b).

During the dissection, prUnipolar and bipolar diathermies were used to minimize bleeding during the dissection procedures. The initial steps of the retrieval surgery were dissection of the bladder peritoneum from the anterior portion of the cervix and mobilization of the bilateral uterine artery and vein, and the deep uterus vein in the pelvic floor (Fig. 2a and b). All blood vessels were doubly ligated (5-0 Vicryl™; Johnson & Johnson, New Brunswick, NJ, USA) or closed by surgical clips (MCATM, Johnson & Johnson). The uterus was cut off between the umbilicus and the intestines were packed into the upper abdomen. The anterior divisions of the uterine vessels, were mobilized from the vagina and severed at a level ≏ 10 mm caudal to the portion of the cervix. The vagina was divided at this level using a monopolar diathermy knife. Blood flow through the uterus was maintained throughout the entire dissection part of the surgery and was retained at the completion of surgery when the uterus was attached only by the bilateral uterine arteries and veins (Fig. 3a). The specimen, including the uterus with a vaginal rim, the oviducts and the ovaries, was removed from the abdominal cavity and put on the backtable (Fig. 3b).

Backtable preparation

During backtable preparation in Cases 3 and 4 (Fig. 4a), the uterus was positioned with its anterior side facing upward and kept in cold saline (4.5°C) during the entire procedure. Although the first two cases were kept at room temperature during backtable preparation, we changed the protocol so that the latter cases were kept cold based on our clinical experience of free jejunum transplantation, in which the ischemic time is a few hours. A plastic surgeon used a stereomicroscope (magnification × 20). To remove blood from the organ, Teflon catheters (inner diameter 0.4 mm; Terumo, Tokyo, Japan) were inserted through the distal ends of the anterior divisions of the internal iliac arteries and advanced into the uterine arteries, and clamps (Sugita Vessel Clip™; Mizuho Medical Co., Tokyo, Japan) were placed around the vessel ends and catheters. The specimen was gently flushed with 20 ml heparinized saline, after which the uterine artery on one side was gently flushed with 30 ml of cold (4°C) University of Wisconsin (UW) solution (Viaspan™; Astellas Co., Tokyo, Japan) (Fig. 4b). The other uterine artery and the uterine veins and deep veins were not ligated to allow drainage. Uterine arteries and veins were not joined.

Autotransplantation

The external iliac artery and vein on the right or left side of the animal were first dissected free over a distance of ≏ 15 mm. The vessels used for anastomosis were selected on the basis of the condition of the
uterine vessels. Vascular clamps were placed on both ends of the isolated segments of the external iliac vein and artery. Incisions were made in the external iliac vessels, and the vein and artery of the specimen were anasto-
mosed end-to-side (Fig. 5a) by interrupted suture (artery, 11-0 nylon; vein, 9-0 nylon; Crownjun, Tokyo, Japan). The vaginal cuff was reattached to the vagina by interrupted suture (5-0 Prolene™; Johnson & Johnson) (Fig. 5b), which was also used to attach the uterine body to the round ligament. After unilateral arterial and venous anastomosis, uterine blood flow was judged to be present based on pulsations of the uterine arteries and capil-
lary refill of the serosal surface of the uterus. Moreover, we performed flow assessment for the uterine artery and vein anastomosis site (Fig. 6a
and b) and blood flow distribution (Fig. 7a and b) using ICG fluorescent angiography immediately after arterial and venous anastomosis. If unstable blood flow was detected, anastomosis of the opposite side uterine artery and vein was performed. The abdomen was closed by continuous sutures of the fascia and skin (2-0 Prolene™; Johnson & Johnson).

ICG fluorescence angiography was performed after unilateral arterial and venous anastomosis. A volume of 0.5 ml ICG (Diagnogreen 0.5%; Daiichi Pharmaceutical, Tokyo, Japan) was injected i.v. via a peripheral venous line. The ICG dye reached the uterine artery ~30 s after the injection. Intravenously injected ICG binds to circulating globulins and remains in the intravascular region. After binding to globulins, ICG absorbs light in the near-infrared range with a maximum of 805 nm and fluoresces with a maximum of 840 nm in plasma. We utilized a newly developed near-
infrared camera system (PDE™; Hamamatsu Photonics KK, Hamamatsu, Japan) that activates ICG with emitted light (wavelength: 760 nm). A surgeon directly handled the camera unit of the device and observed real-
time images on the monitor of a laptop computer.

Assessment of the transplanted uterus using B-Mode or Doppler ultrasonography
At 1, 3 and 6 months after surgery, we checked the blood flow of the transplanted uterus and the presence of hematoma or abscess using B-Mode or Doppler ultrasonography (Fig. 8a and b) and observed the endometrium and uterine size to evaluate the function of the transplanted uterus (Fig. 9). The arterial and venous blood flow rates were determined to evaluate blood flow in the transplanted uterus.
Results

Surgical parameters and time schedule

During the initial dissection studies in two animals, we found that the deep uterine veins (1.0–1.5 mm at 2 cm from the point of its outflow from the uterus) were larger in diameter than the superficial uterine veins (0.3–0.6 mm at 2 cm from the point of its outflow from the uterus). The ovarian veins were also considerably larger in diameter (1.5–2.5 mm at 1 cm from ovary) than the deep uterine veins. However, we did not use the ovarian veins because the uterus and ovary cannot be transplanted en bloc in humans and the ovarian vein cannot be utilized because of the separation of the ovary and uterus. Consequently, we decided to use the uterine artery and deep uterine veins for end-to-side anastomosis with the external iliac veins in the autotransplantation procedure. The background of the four animals that underwent this procedure and the surgical details are shown in Table I. The duration of anesthesia and surgery was 14 h 52 min ± 1 h 45 min (mean ± SEM). The procedure for utero-tubo-ovarian retrieval lasted 3 h 56 min ± 25 min and bleeding was moderate to severe. When the specimen was retrieved, flushing through the single uterine artery ex vivo resulted in an outflow of blood through the bilateral superficial uterine veins and deep uterine veins in all cases. The duration of backtable preparation was 34 ± 20 min.

The duration of anastomosis surgery (Fig. 5a), including the time for the specimen to gradually warm up after cold ischemia, was 4 h 57 min ± 1 h 1 min. The total ischemic (cold plus warm) period lasted for 6 h 54 min ± 1 h 9 min. In all four animals, immediate blood flow through the organ occurred after anastomosis and removal of vascular clamps, with transition of the organ from a whitish to a reddish color, pulsations through the uterine artery and filled outflow veins. After confirming that blood flow had resumed in the transplanted uterus, the bilateral fimbriae of the uterine tubes were fixed to the bilateral ovaries left in the body with two stitches of Vicryl 4-0 (Johnson & Johnson).

Figure 5 (a) Illustration of uterus autotransplantation. The bilateral uterine arteries and deep uterine veins were end-to-side anastomosed with the external iliac arteries and veins, respectively. (b) Intraoperative photograph of uterus autotransplantation. The vagina and uterus were anastomosed by a single interrupted suture using 5-0 prolene.

Figure 6 (a) The uterus after transplantation. The yellow arrow indicates the left uterine arterial anastomosed region. (b) The uterus after transplantation on intraoperative ICG fluorescence angiography. The vascular anastomosed region (yellow arrow) was not narrowed or obstructed and stable blood flow into the uterus was confirmed by ICG fluorescence angiography (see Supplementary Data S1 for video image A).
Intraoperative ICG fluorescent angiography

Blood flow was evaluated in the regions of vascular anastomosis using ICG immediately and 1 h after vascular anastomosis (one artery anastomosis and one venous anastomosis). A thrombus formed ≏15 min after uterine arterial anastomosis in one of the four animals and interrupted uterine arterial blood flow was confirmed using ICG. Uterine arterial anastomosis was repeated and the blood flow improved (Fig. 6a and b, and Supplementary Data S1: video image A). In another animal, the blood distribution in the uterus was found to be unstable using ICG after uterine arterial anastomosis on one side. Therefore, the uterine artery on the other side was anastomosed and the uterine blood distribution was stabilized (Fig. 7a and b, and Supplementary Data S2: video image B). In the remaining two animals, the uterine blood distribution was stabilized by anastomosis of the uterine artery and deep uterine vein on one side and the surgery was completed. In Case 4, after anastomosis and resumed blood flow in the uterine artery on one side, blood first flowed into the whole uterine cervix including the contralateral side, followed by flow into the body of the uterus on the ipsilateral side of the anastomosed uterine artery, and finally into the contralateral uterine body. The entire uterus was finally perfused with blood via a single uterine artery and vein. No adverse effects of ICG, such as allergy, developed in any animal.

Overall outcome and cyclicity

Six cynomolgus monkeys were used in this work, which included two for a dissection study and four for the autotransplantation model. At the time of closing the abdomen (completion of surgery), blood flow...
in the vascular anastomosed regions and transplanted uterus was favorable in all four autotransplantation animals. However, three of the four animals died within 3 months after the operation because of body strength loss and lack of feeding. Although autopsies revealed severe anemia, no intra-abdominal bleeding, intra-abdominal abscesses or pulmonary embolisms were detected. We did not observe necrosis of the uterus or oviducts by gross morphology. The surviving animal resumed cyclicity within 6 months postoperatively and a menstrual stage was observed.

**Assessment of the transplanted uterus using B-Mode or Doppler ultrasonography**

Doppler echo immediately after surgery revealed that uterine arterial and venous blood flows were stable in all four animals that underwent
uteroscopy (Fig. 8a). In the animal that survived for more than 3 months after surgery, the presence of ascites, the endometrium, and uterine arterial and venous blood flow were checked at 1, 3, and 6 months after surgery (Fig. 8b). Although a small volume of ascites was noted immediately after surgery, this disappeared within 1 month. The endometrium was present and the uterine arterial and venous blood flows were observed at 1, 3, and 6 months after surgery. The peak systolic velocity (PSV) of the uterine arterial blood flow immediately after surgery was $19.6 \pm 1.5 \text{ cm/s}$, the end-diastolic volume was $9.1 \pm 0.9 \text{ cm/s}$ and the mean velocity was $13.8 \pm 0.7 \text{ cm/s}$, which were all faster than before the operation. Side differences were observed in two of the monkeys. The PSV was 1.1 times higher in the left uterine artery than the right uterine artery in Case 3 and 1.2 times higher in the right uterine artery than in the left uterine artery in Case 4.

**Discussion**

Herein, we have reported our experience of uterine autotransplantation in four cynomolgus monkeys.

ICG fluorescence uses a near-infrared light source (840 nm) that is highly penetrable in the body, which is advantageous for clear imaging of relatively thin blood vessels. ICG fluorescence was initially used for imaging of retinal blood vessels in fundus photography (Pena-Tapia et al., 2008) and has recently been applied to intraoperative vascular imaging in neurosurgery (Raabe et al., 2005; de Oliveira et al., 2008), cardiovascular surgery (Handa et al., 2009) and liver transplantation (Kawaguchi et al., 2011). Compared with conventional intraoperative X-ray angiography, ICG fluorescence imaging has the advantages of being non-invasive and relatively simple, thus avoiding interruption of surgery. Utilizing these advantages, the patency of anastomosed blood vessels can be checked in real time during surgery and blood perfusion can be assessed. Blood flow can be more reliably managed by measuring the uterine arterial and venous blood flow rates with ICG during surgery. One disadvantage of ICG is that it cannot be used in patients with iodine allergy. However, there were no major adverse effects in this study using monkeys.

Blood flow and the blood-perfused area in the uterus are complex and variation may be present in individuals. It may be necessary to select dominant blood vessels by intraoperatively evaluating blood flow in the donor uterus and the requirement for anastomosis of a second blood vessel can also be judged. The findings from Case 4 showed that unilateral anastomosis of a single artery and vein pair was sufficient for engraftment of the uterus. However, dilation of the transplanted uterus occurs as the uterine content increases during pregnancy and fetal growth and it is therefore unclear whether blood flow through a unilateral anastomosis of the uterine artery and vein will be sufficient. This issue remains to be investigated.

Regarding selection of a venous blood vessel for uterus transplantation, Enskog et al. (2010) used the uterine artery and ovarian vein for uterus transplantation in baboons, because the ovarian vein was the thickest among the uterine veins. Although this is also true of the cynomolgus monkey, the use of the ovarian vein is not realistic for application of uterus transplantation in humans. We therefore used the deep uterine vein with the goal of mimicking the clinical application of uterus transplantation in humans as closely as possible.

Disadvantages of this procedure include difficult vascular dissection in the pelvic floor and hemostasis once bleeding occurs.

Uterus transplantation is a complex procedure and three out of four of our cases died within 3 post-operative months: the biggest contributing factor was loss of physical strength after a long operation. It may be necessary to select a relatively large individual and shorten the operation time. We are also investigating approaches for increasing the transplantation success rate through improved intra- and post-operative management, such as treatment with blood transfusion or the use of a hyperoncotic solution that contains a high concentration of hydroxyethyl starch.

We attempted transplantation of oviducts with fimbriae with the uterus in Case 2. In monkeys, IVF and transovuductal embryo transfer prevail because the cervical duct is long and tortuous and transvaginal embryo transfer is difficult. Furthermore, we also expected natural pregnancy. The possibility of extraterine pregnancy may increase and further investigation on this topic is needed.

We conclude that evaluation of intra- and post-operative blood flow in the transplanted uterus will help increase the success rate of uterus transplantation. ICG fluorescence angiography can be used for simple real-time assessment of the blood flow conditions of the uterus. Intraoperative evaluation of dominant blood vessels in individual uterine grafts using ICG fluorescence angiography may lead to the establishment of a more stable uterus transplantation procedure.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/.

**Authors’ roles**

M.M. collected data and wrote the manuscript. T.I., H.H., T.Y., J.A., Y.H., H.M., M.N., K.B., N.S., D.A. and Isao Koshima collected and analyzed data.

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