Fluorescence Video Angiography for Evaluation of Patency and Patent Artery Integrity in a Preclinical Experimental Setting

Basil E. Grüter, MD[1,2], Dominik Täschler, MSc[2], Jeannine Rey[2], Fabio Strange, MD[1,2], Edin Nevzati, MD[2], Javier Fandino, MD,[1,2], Serge Marbacher, MD, PhD.[1,2], and Daniel Coluccia MD[1,2]

[1] Department of Neurosurgery, Kantonsspital Aarau, Aarau, Switzerland

[2] Cerebrovascular Research Group, Department for BioMedical Research, University of Bern, Bern, Switzerland

Portions of this work have been presented:

- For JOVE "Film Your Research Contest", online, June 15, 2017 (in video form): (https://www.youtube.com/watch?v=Sc_0VzTMAGk)
- 21st Annual Meeting of the Swiss Stroke Society (SSS), January 11, 2018, Lausanne, Switzerland (poster)
- Joint Annual Meeting Swiss Society of Neurosurgery (SSNS) and Swiss Society of Neuroradiology (SSNR), May 24-25, 2018, Lugano, Switzerland (poster)

Source of funding

This study was supported in part by a research grant of Kantonsspital Aarau, Switzerland (FR 1400.000.054) The operation microscope used was sponsored by Carl Zeiss AG, Oberkochen, Germany.

Disclosure of interest

The authors report no disclosures or conflict of interest

Acknowledgments

We thank Marco Necchi, RN, for technical support and Boris le Feber for advice in questions related to light physics. We are thankful to Carline Perren for her help with the administrative burden. We acknowledge Mary Kemper of Glia Media for editing.

Corresponding author: Basil E. Grüter, MD, Department of Neurosurgery c/o Neuro Research Office (NRO), Kantonsspital Aarau, Tellstrasse 1, 5001 Aarau, Switzerland, +41/ 062 838 66 34
Email: mailto: basil.grueter@ksa.ch; neurosurgery@ksa.ch

Abstract

Background: Experimental studies to assess aneurysm occlusion or perfusion typically rely on macroscopic examination or histological analysis but cannot assess dynamic perfusion.

Objective: This study describes an easy to implement and inexpensive fluorescence angiographic technique for *in vivo* assessment and imaging of dynamic perfusion status of aneurysms and their underlying blood vessels in a rat model.

Methods: In a rat sidewall aneurysm model, the angiographic setup included 2 bandpass filters, a video camera, and bicycle spotlight. After 48 rats underwent fluorescein angiography, dissections were performed to confirm perfusion status by macroscopic and histologic examination of the aneurysm.

Results: Direct injection of 0.2 ml fluorescein 10% Faure achieved strong, clear visibility in all 48 aneurysms. Macro-/microscopic examination identified residual perfusion in 25 and complete healing in 23 aneurysms. Fluorescein imaging identified 21 of these 25 aneurysms (84%) with residual perfusion and 22 of 23 aneurysms (96%) with no residual perfusion.

Conclusions: Our fluorescein imaging technique proved efficient for the evaluation of aneurysm patency and parent artery integrity in this experimental setting. Fluorescein is non-toxic, can be re-administered if needed, and, in this technique, can expand the armamentarium for pre-clinical evaluation of dynamic perfusion status.

Key Words: Aneurysm; Experimental; Fluorescein; Fluorescence; Rat; Video angiography

Running title: Fluorescence Video Angiography in the Rat model

Introduction

Cerebral aneurysms are flow-dependent vascular lesions. Assessment of perfusion status and visualization of arterial patency is crucial in the management of cerebral aneurysms in clinical settings and pre-clinical research in animal models. Several imaging techniques, such as Doppler ultrasonography (US), computed tomography angiography (CTA), magnetic resonance angiography (MRA), or digital subtraction angiography (DSA) have been established to monitor treatment of these flow-dependent vascular lesions.[1] However, these methods usually require complex and expensive apparatus, which may not always be available or affordable. Besides being time consuming, these imaging modalities can be associated with substantial side effects (e.g., radiation exposure). As a result, experimental studies of new devices (e.g., coils, stents) to determine the extent of aneurysm occlusion or perfusion typically rely on macroscopic examination and histological analysis. However, this does not allow the assessment of dynamic perfusion status.

One different, inexpensive, and simple method to image intraluminal blood flow is fluorescence angiography, using either near-infrared indocyanine green (ICG) or fluorescein. Both techniques require direct aneurysm exposure. ICG angiography is a well-established technique in various surgical fields[2] and routinely used in neurosurgery to visualize blood flow during cerebral aneurysm surgery.[3-5] Fluorescein angiography, which is mainly used in ophthalmology to visualize blood vessels in the retina and choroid coat,[6,7] has also been suggested as an useful adjunct to cerebral aneurysm surgery.[8-10] In contrast to ICG, an advantage of fluorescein is that the light emitted by excited fuorescein occurs within the range of human vision (380 nm to 780 nm) and thus can be seen without a camera.

This study describes our fluorescence angiographic technique for *in vivo* assessment and imaging of the dynamic perfusion status of the aneurysms and their underlying blood vessels in a rat model. The technique used a simple setup that consists of two off-the-shelf bandpass filters, camera, and bicycle spot light.

Methods

Microsurgical Aneurysm Creation

Male Wistar rats (Janvier Labs, Le Genest-Saint-Isle, France) were housed in an animal facility at a room temperature of 22-24°C and 12-hour light-dark cycle, with free access to tap water and food. Animals received humane care in accordance with institutional guidelines.

Experiments used the Helsinki rat aneurysm model as previously described.[11] Briefly, a standardized piece of the descending thoracic aorta of a donor animal ligated and sutured to the infrarenal abdominal aorta of a recipient animal formed an aneurysm. The experiments were approved by the Committee for Animal Care of the Canton Bern, Switzerland as part of an ongoing study investigating the role of immunomodulation on aneurysm healing (BE 65/16).

Excitation Setup

We used Fluorescein 10% Faure (0.5 g/5 ml), an inexpensive and easy obtainable fluorescein sodium solution. The excitation wavelength of fluorescein ranged from 465 nm to 490 nm; its emitted fluorescence signal was observed at wavelengths from 520 nm to 530 nm.[6] A common bicycle LED spotlight (LED -luminous flux of 1200 lumen, power 10W, powered by an accumulator with 4400 mAh and a continuous light of 1200 LM, at costs of 39.-$) was the excitation light source. Since the light emitted by the white-light LED also included wavelengths within the transmission spectra of the fluorescein sodium, an excitation bandpass filter attached to the spotlight avoided interferences with the light emitted by the fluorescein sodium. The excitation filter (Thorlabs MF475-35, Newton, New Jersey, USA) had its center transmission wavelength at 475 nm and provided a full width at half maximum (FWHM) bandwidth of 35 nm and costs of 242.-$. Ideal distance between the illumination source and the light emitting blood vessel or aneurysm is approximately 30cm.

Detection Setup

The surgical microscope (OPMI®, Carl Zeiss AG, Oberkochen, Germany) provided two separate light channels, which can be expanded with additional optical devices. During examination, one channel was used for fluorescence video registration. To ensure that only light emitted by the excited fluorescein would be detected by the camera (Sony NEX-5R, Tokio, Japan), a detection bandpass filter (Thorlabs MF530-43 Newton, New Jersey, USA) was placed in front of its objective lens (Figure 1). This filter type transmitted only light within a defined wavelength range, that is, the light emitted by the fluorescein sodium. The emission filter had a center transmission wavelength of 530 nm and a FWHM bandwidth of 43 nm and costs of 242.-$.

Excitation and detection hardware can physically be united in a body that combines a white light source (plus excitation filter) and a digital camera (behind a detection filter). A basic smart phone is ideal a body with these properties (Figure1 and 2).

Aneurysm exposure

To visualize blood flow with fluorescence angiography, in n=48 animals the relevant blood vessel or aneurysm was fully exposed to a light source. After careful dissection of the aneurysm, the femoral vein was dissected to select an injection site for the fluorescein sodium (as described elsewhere).[12] In brief, following skin incision distal and in parallel to the inguinal ligament, the fat and muscle tissues along the cutting edge were dissected to identify the femoral vein in the deep (Figure 3).

Execution of the FA

After the fluorescein sodium (0.2 ml/500 mg fluorescein 10% Faure) was slowly injected into the rat's femoral vein, the aneurysm was exclusively illuminated by the excitation beam of the LED after a few seconds, presumably the time needed for the medicament to be perfused from peripheral application site to the centrally located aneurysm on the abdominal aorta. Interference signals were eliminated by turning off all light sources (i.e., microscope or room light) during fluorescence imaging. When necessary, a red light was used because it did not interfere with the light emitted by the fluorescein sodium. The emitted light signal was detected by eye and simultaneously filmed for documentation (Figure 4). The light emitting effects holds on for several minutes, before slowly fading out.

Results

After evaluation by fluorescence angiography *in vivo*, all 48 aneurysms were dissected and macroscopically and microscopically examined to ultimately determine the actual perfusion status. Macro-/microscopic examination identified residual perfusion in 25 of these 48 aneurysms as determined by incomplete neointima formation over the aneurysm orifice; fluorescein examination detected residual perfusion in 21 of these 25 aneurysms (84%). Among the 4 false-negative cases, 3 aneurysms had little to minimal residual blood flow, which was located in the middle of the aneurysm orifice in all 3 cases. Therefore, a relatively thick layer of scar tissue was around the remaining lumen where the residual blood flow was seen. Subsequently, the light emitted had a relatively thick tissue barrier to pass. In the fourth case, the tissue layer was too thick for the light to pass because of insufficient dissection; too much connective tissue persisted around the aneurysm's outer wall and the underlying vessel.

The remaining 23 aneurysms were completely healed with no residual perfusion detected by macroscopical evaluation. Fluorescence angiography correctly identified 22 of these 23 aneurysms (96%) with no residual perfusion. In 1 case, aneurysm exposure provoked microbleeds that led to micro-leakage of blood (containing fluorescence) on the aneurysm's outside and subsequent contamination. Therefore, fluorescence signal was positive despite no true blood flow inside the aneurysm. The underlying artery was patent in all 48 aneurysms and correctly visualized by fluorescence angiography in all cases.

After successful direct injection of 0.2 ml fluorescein 10% Faure, visibility was strong and clear in all 48 animals, and no second injection was administered. Injection of 0.1 ml fluorescein as tested in preceding pilots was technically much more difficult (due to venous back flow of a few droplets at the injection site) and had to be repeated to result in strong visibility by angiography. See video1 for demonstration of the whole procedure.

Discussion

In this rat (sidewall) model, fluorescein video angiography provided an easy, inexpensive technique to assess dynamic blood flow within the experimental aneurysm and patency of the parent vessel. The varying light emitted depended on the fluorescein sodium concentration in the blood. Because it is non-toxic, fluorescein could be re-administered if the signal weakened or a repeat examination was needed. Our technique's other main advantage was that almost any ordinary light source could be used for excitation with a bandpass light filter, for example, a smartphone equipped with specific excitation and detection filters. As the digital detection camera is not specific, neither, a phone camera can equally be used for video recording in combination with the detection filter.

Imaging of perfusion and patency in experimental models

Although many studies have reported various techniques to image aneurysms in humans, few methods have been specifically developed for a pre-clinical setting. To the best of our knowledge, of only two studies that used fluorescence angiography after microsurgical procedures in rats, neither discussed the topic of aneurysm perfusion status. In the first study evaluating microvascular anastomoses in rats, Mucke et al. reported that ICG (in combination with microvascular Doppler) was a quick and reliable method for assessing blood flow in microvascular anastomoses in rats; its rapid clearance allowed repeat assessments every 10

minutes.[13] We did not use ICG because its signal wavelengths of 820-900 nm are imperceptible to the human eye.[12] Additionally, standard cameras often have an internal shortpass-filter that excludes these wavelengths. Table 1 compares further properties of ICG and fluorescein.

In the second 1974 study of fluorescein angiography, Fox and Yasargil used an intra-arterial injection of 0.5 ml of 5% fluorescein to detect vessel injuries after microvascular anastomoses in rats.[8] In comparison, we used a 10% concentration that reduced venous injection volumes to 0.2 ml/500g. Technical equipment for imaging was far more laborious in the 1970's and video documentation was cumbersome. Nonetheless, physical principles observed have remained consistent throughout the decades.

Fluorescence is primarily an adjunct technique which allows for quick intraoperative evaluation of a dynamic perfusion status in a vessel or a vessel pouch (aneurysm). Therefore, its main application in the operating room has different requirement in a clinical and in a preclinical setting. Ichikawa et al. described a filter system which can be attached to an operating microscope without a fluorescence mode, to enable fluorescence angioraphy.[5] For fixation of the filter system an auxiliary tool is needed which will then allow to switch filters in front of the microscope white light source. We preferred an additional (external) light source because normally operating microscopes already work with a set of internal lights filters to focus the emitted white light and to prevent optical parts from overheating. Thus, to install an additional filter (to obtain the blue light needed for fluorescence excitation) - in a way which enables the operator to quickly change between with light and fluorescence mode is relatively laborious. The use of a smart phone instead is much easier to handle, avoids mechanical systems for switching between filters and is still adequate in a preclinical setting.

Excitation Bandpass Filter and Detection Signal

A main advantage for our technique was excitation achieved by an ordinary light source coupled with a bandpass light filter, permitting only the desired excitation wavelength (465-490nm) to reach the sample while blocking wavelengths above and below this range. With availability of a specific bracket, the bandpass filter can be installed directly onto the microscope. Alternatively, excitation can be achieved with an external white light source equipped with a specific bandpass filter (as in our experiment) or an appropriate monochromatic laser source (emission wavelength range 465 to 490 nm). For signal detection, light emitted by fluorescein lies within the range of human vision (380-780 nm) and is also detectable by most commercial

cameras (e.g., compact cameras, smartphones). Basic models of digital cameras are available for less than 100.-$ these days. For example, a smartphone equipped with a set of excitation and detection filters was sufficient technical equipment to perform fluorescence video angiography (Figure 1B).

Pitfalls in Signal Strength

Fluorescein sodium is a nontoxic, inexpensive fluorescence dye available as powder or premixed solution. Given the light emitted depends on the fluorescein sodium concentration in blood, the dye's concentration should not be changed during an experiment and dosage should be adopted based on the animal's weight (or estimated blood volume). After intravenous injection, fluorescein is quickly glucuronidated to fluoresceinglucuronid, which also has fluorescent properties. In plasma, fluorescein and fluoresceineglucuronid have a terminal half-life of 23.5 and 264 minutes, respectively (table 1). Thus, after one application, the light-emitting effect remains strongly visible for several minutes before fading out. Because fluorescein is non-toxic, it can be re-administered if the signal weakens too much or if a second examination is needed later. As detected signal strength of fluorescein depends on many factors (such as gluocoronidation and consecutive signal weakening) as well as vessel wall thickness as a physical barrier for the emitted light) quantification of the blood flow is not reliably possible. Therefore, fluorescence angiography is rather ideal to dichotomize between perfusion (open vessel) vs. no blood flow (obliteration).

To ensure a proper and strong fluorescence signal, the aneurysm and its underlying artery must be widely separated from surrounding connective tissue. Insufficient dissection will likely obscure fluorescence signal, which may lead to false-negative results (e.g., no apparent blood flow where there is in reality). Besides clean dissection, meticulous haemostasis is crucial before fluorescein injection because even minor bleeding can result in dye extravasation and contamination of the outer aneurysm tissue, which may mimic a false-positive result. As the examined vascular tissue needs to be fully exposed, fluorescence angiography is ideally performed during surgery. For routine visualization, however, in a living animal, other, less invasive imaging techniques may be preferable.

Venous injection site

Fluorescein sodium can be injected in any vein of strong enough calibre for cannulation. Our use of the femoral vein ensured a spatial distance from injection site to the aneurysm in the rat's abdominal cavity. This prevented contamination of the aneurysm site by fluorescein sodium leakage from the injected vessel. Other veins (e.g., tail vein) may represent an equally suitable alternative injection site, with the idea of creating only a minimal injury on the animal, particularly if the examination is performed earlier to final follow-up.

Conclusion

Fluorescein video angiography in our experimental setting was performed with minimal equipment that included an excitation and detection filter in combination with a white light source and detection camera. Fluorescein video angiography was an easy, inexpensive technique to assess dynamic blood flow within experimental aneurysms and parent vessel patency in a rat (sidewall) aneurysm model.

References

1. Ajiboye N, Chalouhi N, Starke RM, Zanaty M, Bell R. Unruptured Cerebral Aneurysms: Evaluation and Management. TheScientificWorldJournal. 2015;2015:954954.
2. Alander JT, Kaartinen I, Laakso A, et al. A review of indocyanine green fluorescent imaging in surgery. Journal of Biomedical Imaging. 2012;2012:7.
3. Raabe A, Nakaji P, Beck J, et al. Prospective evaluation of surgical microscope-integrated intraoperative near-infrared indocyanine green videoangiography during aneurysm surgery. Journal of neurosurgery. Dec 2005;103(6):982-989.
4. Hodge CP, Yamamoto YL, Feindel W. Fluorescein angiography of the brain--the photographic procedure. Journal of the Biological Photographic Association. Apr 1978;46(2):67-79.
5. Ichikawa T, Suzuki K, Watanabe Y, et al. Development of and clinical experience with a simple device for performing intraoperative fluorescein fluorescence cerebral angiography: technical note. Neurologia medico-chirurgica. 2016;56(3):141-149
6. Bennett TJ, Quillen DA, Rolly Coronica D. Fundamentals of fluorescein angiography. Curr Concepts Ophthalmology. 2001;9(3):43-49.
7. Flower RW. Injection technique for indocyanine green and sodium fluorescein dye angiography of the eye. Investigative ophthalmology. Dec 1973;12(12):881-895.
8. Fox JL, Yasargil MG. Fluorescein angiography in microvascular surgery: a study using the rodent artery. Stroke; a journal of cerebral circulation. 1974;5(2):196-206.
9. Kuroda K, Kinouchi H, Kanemaru K, et al. Intra-arterial injection fluorescein videoangiography in aneurysm surgery. Operative Neurosurgery. 2012;72(suppl_2):ons141-ons150.
10. Suzuki K, Kodama N, Sasaki T, et al. Confirmation of blood flow in perforating arteries using fluorescein cerebral angiography during aneurysm surgery. Journal of neurosurgery. Jul 2007;107(1):68-73.
11. Marbacher S, Marjamaa J, Abdelhameed E, Hernesniemi J, Niemela M, Frosen J. The Helsinki rat microsurgical sidewall aneurysm model. Journal of visualized experiments : JoVE. Oct 12 2014(92):e51071.

12. Geishauser L. Microsurgery Interactive Video Learning Modules. Rat femoral artery model microsurgical dissection http://microsurgery.video/index.php/Rat-femoral-artery-model-table-setup.html.
13. Mucke T, Wolff KD, Wagenpfeil S, Holzle F, Scholz M. Reliability of near-infrared angiography and micro-Doppler sonography for evaluating microvascular anastomoses. Plastic and reconstructive surgery. Nov 2010;126(5):1506-1514.

Figures

Figure 1: Settings used for fluorescence angiography: (A) Set-up with an external camera mounted on a microscope camera adapter (*). Detection bandpass filter (**) is fixated between the camera and microscope. (B) Alternative smartphone set-up with visible excitation (*) and detection filter (**) fixed on the phone.

Figure 2: Intraoperative fluorescence video angiography with the smart phone setting. Note, camera and light are both on the backside of the phone (not visible). Ideal distance between the phone and the vessel is approximately 30cm.

Figure 3: Fluorescein dye injection site in the femoral vein. Anatomical landmarks are: 1) femoral vein, 2) popliteal vein, 3) greater saphenous vein, 4) inguinal ligament

Figure 4: White light image and video snapshots of fluorescein angiography with delineated aneurysm and parent vessel. The operative situs in white light through the operation microscope (A) shows a dissected aneurysm. Below the underlying parent artery, a rubber pad (green) is placed to separate the artery form the cava vein and the retroperitoneal tissues. In (B) fluorescence angiography of a fully perfused aneurysm is shown, right after its creation (dotted line marks the aneurysm border). After several weeks of treatment, substantial residual perfusion (C) is seen in the neck and dome of the aneurysm with strong blood flow in the underlying vessel,

compared to a completely healed aneurysm (D) with no residual perfusion in the aneurysm but patent underlying vessel (micro forceps from the left side with the tip on the aneurysm).