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Methods in eye research

Murine fundus fluorescein angiography: An alternative approach using a handheld camera

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ABSTRACT

In today's modern pharmacologic approach to treating sight-threatening retinal vascular disorders, there is an increasing demand for a compact, mobile, lightweight and cost-effective fluorescein fundus camera to document the effects of antiangiogenic drugs on laser-induced choroidal neovascularization (CNV) in mice and other experimental animals. We have adapted the use of the Kowa Genesis Df Camera to perform Fundus Fluorescein Angiography (FFA) in mice. The 1 kg, 28 cm high camera has built-in barrier and exciter filters to allow digital FFA recording to a Compact Flash memory card. Furthermore, this handheld unit has a steady Indirect Lens Holder that firmly attaches to the main unit, that securely holds a 90 diopter lens in position, in order to facilitate appropriate focus and stability, for photographing the delicate central murine fundus. This easily portable fundus fluorescein camera can effectively record exceptional central retinal vascular detail in murine laser-induced CNV, while readily allowing the investigator to adjust the camera's position according to the variable head and eye movements that can randomly occur while the mouse is optimally anesthetized. This movable image recording device, with efficiencies of space, time, cost, energy and personnel, has enabled us to accurately document the alterations in the central choroidal and retinal vasculature following induction of CNV, implemented by argon-green laser photocoagulation and disruption of Bruch's Membrane, in the experimental murine model of exudative macular degeneration.

1. Introduction

Murine fluorescein fundus angiography (FFA) has taken upon great importance with the emergence of pharmacologic therapies replacing laser photocoagulation as the mainstay of treatment for many retinal vascular disorders (Brown et al., 2009). Since laser photocoagulation can be used to disrupt Bruch's Membrane to induce an experimental model of choroidal neovascularization (CNV) in mice (Tobe et al., 1998), murine FFA can be used to monitor the effects of various new pharmacologic agents that may have the potential to block different inflammatory (Edelman et al., 2005; Chen et al., 2012; Parmeggiani et al., 2012), or angiogenic (Benny et al., 2008; Michels and Rosenfeld, 2005; Krzystolik et al., 2002; Nork et al., 2011) signals in the formation of human CNV, in age-related macular degeneration (ARMD).

Since the mouse eye has developmental rapid increases in its axial length and its corneal radius of curvature (Zhou et al., 2008), an additional objective convex lens must be supplemented to a human fundus camera, in order to produce clear and accurate retinal images of the murine retina, in compensating for the additional hyperopia of the mouse eye, compared to the human eye. In 1994, DiLoreto and associates (DiLoreto et al., 1994) used a 2.2 Volk Panretinal lens that was held in apposition to the lens of the Topcon TRC 50T clinical fundus camera, by means of a custom-made metal sleeve. Similarly, Cunea and colleagues (Cunea et al., 2014) described the use of a custom-made clamping device to connect the same 2.2 Volk lens in front of a Zeiss FF450plus clinical fundus camera. Finally, Marneros and collaborators (Marneros et al., 2007) placed a +7.00 diopter contact lens on the mouse cornea, in conjunction with their Topcon TRC50-IA clinical fundus camera.

Similarly, as occurred in human FFA, murine FFA progressed from recording on analog camera devices that utilized 35 mm ASA,

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black and white 400 film (DiLoreto et al., 1994; Okamoto et al., 1997; Hawes et al., 1999) to more elaborate equipment that has included computerized digital imaging (Cunea et al., 2014; Marneros et al., 2007; Benny et al., 2010). In most cases, as takes place in clinical FFA, the computerized digital FFA appliances used in murine FFA, are large and only, by example, weigh from the Heidelberg HRA2 Scanning Laser Ophthalmoscope (Benny et al., 2010) which measures 19.1 kg, to the Topcon TRC50- IA (Marneros et al., 2007) digital fundus camera which registers at 35 kg. As a result, these devices consume a considerable area of space, usually remain stationary in location, and as takes place in human FFA, the research subject in rodent FFA, practically, must be brought to the dedicated location of these bulky photographic appliances.

Finally, with advancements in retinal photography and computerized operations, the fundus devices became compact for clinical and veterinary research utilizations. For example, Kowa developed the Genesis-D Hand-Held Retinal Camera for color photography of the posterior segment, which has been described for human use (Ozerdem, 2009), as well as applications for murine research (Hawes et al., 1999), that is designed for mydriatic color fundus photography for animal research, as well as for clinical applications in patients, especially those elderly or disabled individuals, confined to a domiciliary location.

We now introduce a different FFA approach utilizing the recently manufactured Kowa Genesis Df Retina Camera (Kowa Company, Ltd., Tokyo, Japan) that presents a lightweight (1.070 kg), freely mobile, and handheld compact (27.85 cm high) digital camera that can effectively record and perform FFA on experimental rodents, rabbits and primates, as well as on bedridden patients in clinical practice.

2. Materials and supplies

2.1. Supplies

2,2,2-Tribromoethanol (Avertin, Sigma-Aldrich, St. Louis, MO., U.S.A., Ref. T48402). Cyclopentolate HCl 1% ophthalmic solution (Midriodavi, Povoa de Santo Adriao, Portugal). Phenylephrine HCl 10% ophthalmic solution (Efrin-10, Fischer Labs, Tel-Aviv, Israel (IL.)). Carbomer 2 mg/g oph. gel (Viscotears, Dr. Mann, Germany; for: Novartis, Basel, Switzerland). Micro cover glass 18 × 18 mm, cat. No. 48366-205 (VWR, vwr.com, Radnor, PA, U.S.A.) Sodium fluorescein 2% (Sigma-Aldrich, St. Louis, MO., U.S.A.).

Povidone 1.6%, Hydroxyethylcellulose 0.31% oph. sol.(Lacrimol, Fischer Labs, Tel-Aviv, Israel).

2.2. Equipment

Nidek argon-green (532 nm) photocoagulator (GYC2000, Nidek, Osaka, Japan); Kowa Genesis Df Handheld Retinal Camera (Kowa Company Ltd., Tokyo, Japan); Kowa Genesis Df Indirect Lens Main Unit Holder Arm (Part no. K9L-LH51C) (Kowa, Japan); 90 Diopter clear lens (OI-STD 90D, Ocular Instruments, Inc., Bellevue, WA, U.S.A.); Compact Flash Type I, FAT-format, 2 GB memory card (SanDisk, Milpitas, CA, U.S.A.); USB 3.0 multi-format memory card reader (Insignia, Best Buy, Richfield, MN, U.S.A.); Acrylic platform: 240 mm × 180 mm × 8 mm (Custom-Made, Hebrew University Engineering; Givat Ram, Israel); Acrylic rim: 10 mm × 100 mm × 5 mm: On central platform (Custom-Made, Hebrew University Engineering, Givat Ram, Israel).

2.3. Experimental animals

Animal experiments of the proposed projects were carried out in accordance with the guidelines and with the approval of the relevant authorities of the Institutional Animal Care and Use Committee of Hebrew University of Jerusalem, Israel, with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978), and with the ARRIVE guidelines. Eight week old C57BL/6 mice were purchased from Harlan Laboratories Ltd. (Jerusalem, Israel).

3. Detailed methods

3.1. Induction of murine choroidal neovascularization (CNV)

In our investigations, after the mouse was sedated with intraperitoneal (i.p.) tribromoethanol (Avertin) (Papaioannou and Fox, 1993), 0.25 mg/g, its pupils dilated with cyclopentolate 1% and phenylephrine 10% ophthalmic drops, and placed on an acrylic platform, as described in section 3.2, it was then placed in front of a Nidek GYC-2000 532 nm Photocoagulator (Nidek, Inc., Osaka, Japan). After placing a micro cover glass (VWR, vwr.com, Radnor, PA, U.S.A.) with Carbomer ophthalmic gel (Novartis, Basel, Switzerland) onto the mouse cornea, the argon-green laser beam was aimed at the central retina, temporal to the murine optic disk, or at the murine peripapillary region, and using a 50 μm spot size, at 0.1 s duration, and 200 mw intensity, a subretinal gaseous bubble was formed at the level of Bruch's Membrane.

3.2. Murine fundus fluorescein angiography (FFA) with Kowa genesis Df camera

One week following induction of CNV in mice, the Kowa Genesis Df Handheld Retinal Camera (Kowa Company Ltd., Tokyo, Japan) (Fig. 1A), with an incorporated blue excitation filter with an excitation wavelength of 490 nm and an implanted barrier filter with an emission wavelength of 525 nm, was prepared and utilized to capture individual murine fundus fluorescein angiographs (FFA), by

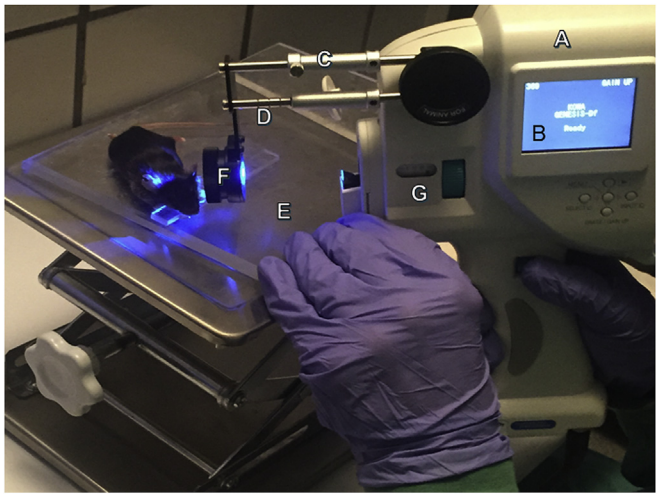

Fig. 1. The Kowa Genesis Df Fundus Fluorescein Angiography (FFA) Handheld Retinal Camera. (A) An investigator is holding the compact and lightweight Genesis Df camera (connected peripherally with a separate cable to a footswitch shutter). (B) L.E.D. Screen display for calibrating settings for the camera, and for visualizing the latest angiograph. (C) The securely attached main unit holder with holder arm for the 90 diopter lens. (D) In order to have the clearest focus of the central murine retinal and choroidal vascular network, the main unit holder arm is adjusted to be secured to the fourth notch of the sliding bar, that is directly below and parallel to the metal cylinder and lens holding arm labeled as C. (E) This sedated mouse is comfortably leaning its mandible on the acrylic rim of the acrylic platform. (F) The 90 diopter lens is securely fixed inside the 90 diopter lens holding ring. (G) In order to maximize focus, the vertical dial is adjusted.