FULL PAPER *Surgery*

Indocyanine Green Angiography for Examining the Normal Ocular Fundus in Dogs

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ABSTRACT. In dogs, a variety of diseases of the retina and choroid have been reported, either separately or concomitantly; however, the canine choroid is difficult to evaluate by veterinary techniques currently available. Indocyanine green (ICG) angiography is widely used in human ophthalmology, but has not been investigated for use in canine ophthalmology. The aim of this study was to apply a new approach to ICG angiography and compare the resulting angiograms with fluorescein (FLUO) angiograms of the ocular fundus in dogs. With a fundus camera equipped with an infrared-sensitive charged coupled device (CCD), we performed angiography on eight healthy beagles under inhalation anesthesia. ICG angiography enabled clear visualization of the choroidal vasculature, whereas FLUO angiography showed only the retinal vessels. At 8.4 ± 3.6 sec after administration of ICG dye into the cephalic vein, the choroidal arteries could be seen extending radially from the optic disc, then the choroidal veins became apparent at 10.2 ± 4.1 sec, coursing alongside the choroidal arteries. Gradual fading of the choroidal vessels began 13.2 ± 2.2 min after the dye was administered, and overall diffuse fluorescence of the fundus appeared. Diffuse fluorescence of the fundus continued after the choroidal vessels and optic disc faded at about 58.3 ± 5.3 min from administration of the dye. In conclusion, ICG angiography provides clear resolution and is reliable and simple, thus offering promise as a diagnostic aid for clinical evaluation of the choroid in dogs.
KEY WORDS: choroid, dog ophthalmology, fluorescein, indocyanine green, ocular angiography.

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In the dog eye, retinal circulation can be examined with clarity, but by current veterinary techniques the choroidal circulation eludes examination [7]. Consequently, disorders associated with the choroidal vessels could go undetected until it is too late to ward off deterioration of the animal's vision. Situated between the sclera and the retina, the choroid is a strategic part of the vascular tunic that provides nutritive support to the retina. Given that early recognition of possible ocular pathologic change is paramount to maintaining the eyes in working condition, a new diagnostic approach is needed for the imaging of the vascular structures of the choroid.

For examination of retinal circulation, fluorescein (FLUO) angiography is currently used in both human and animal ophthalmology. As a diagnostic tool, the FLUO angiogram provides a reliable guide for laser photocoagulation of exudative and proliferative retinal diseases such as diabetic retinopathy or occlusion of the retinal branch vein [22]. Poor transmission of fluorescence, however, is an inherent characteristic of FLUO dye that limits its usefulness in evaluating either the normal or abnormal choroidal vessels. FLUO dye does not allow visualization through the ocular media opacification, the fundus pigmentation, or through pathologic manifestations [1].

In human ophthalmology, indocyanine green (ICG) dye has gained popularity as either an adjunct or alternative to FLUO, particularly because of the optical and biophysical properties of the ICG dye. In contrast to FLUO, the near-infrared light absorbed and excited by ICG readily penetrates normal ocular pigments [24]. Furthermore, approximately 98 percent of the ICG dye binds rapidly to blood proteins, thereby curtailing escape of the dye through the fenestrations of the choriocapillaris [2]. Because of these properties, ICG angiography permits better visualization of the choroidal vessels than FLUO angiography. The molecules of the FLUO dye do not bind so readily to the blood proteins and, consequently, pass freely out of the choriocapillaris, thus obscuring the choroidal details.

In the early years of human angiography using ICG, the low sensitivity of monochrome infrared film presented difficulties and the fluorescence intensity of the ICG dye in blood was approximately 25 times lower than that of FLUO dye [2, 21]. The ICG technique was modified by Hayashi *et al.* with the use of videoangiography employing an infrared-sensitive charged coupled device (CCD) as the means of recording the low fluorescence of ICG [11]. Subsequently, ICG angiography has now been widely used for 20 years and has proven useful in diagnosing human choroidal diseases that were not disclosed before, such as age-related macular degeneration with subretinal neovascularization, polypoidal choroidal vasculopathy resulting in hemorrhagic retinal detachment, and Vogt-Koyanagi-Harada syndrome with choroid inflammation [16, 19, 23].

In dogs the choroid is obstructed by the shielding effect of the tapetum and by diffuse pigmentation in the ocular fundus. We hypothesized that ICG angiography employing the infrared-sensitive CCD would make it possible to assess the

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choroidal circulation and thus diagnose choroidal diseases in dogs.

The aim of the present study was to evaluate the capability of ICG angiography in comparison to FLUO angiography of the ocular fundus in healthy beagles.

MATERIALS AND METHODS

Animals: In 8 healthy laboratory beagles, angiographic examinations were performed in 10 eyes. The dogs ranged in age from 3 to 9 years (median: 4.6 years), and their body weight was 9.2 to 12.6 kg (median: 10.8 kg). All animals had a normal ocular fundus with abundant reflection from the tapetum and normal pigmentation of the non-tapetum, as shown by indirect ophthalmoscopy. The study protocol was approved in advance by the Animal Care Committee of Rakuno Gakuen University.

Angiographic fundus camera: For both ICG and FLUO angiography, we used a fundus camera (TRC50IX; Topcon, Tokyo, Japan) fitted with an infrared-sensitive CCD. For ICG angiography the fundus camera had an excitation filter at 800 nm with a barrier filter ranging from 780 to 805 nm, and for FLUO angiography the fundus camera had an excitation filter at 490 nm and a barrier filter at 520 nm. Digital fundus photographs taken with the Topcon IMAGEnet 2000 system were recorded on videotape. Angiographic photographs of each fundus were taken at both 50-degree and wide-angle fields of view. The wide angle technique, a modification of the method described by Sutoh *et al.* [20], was achieved by use of the fundus camera in conjunction with a 20 Diopter lens (Indirect lens; Lumenis, Tokyo, Japan).

Preparation of the dog for angiography: The pupil was dilated with 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P; Santen, Osaka, Japan) prior to angiographic examination. All dogs were anesthetized with oxygen-sevoflurane (Sevofrane; Maruishi, Osaka, Japan) by use of a mask and orotracheal intubation, and anesthesia was maintained on inhalation of oxygen-sevoflurane. We administered pancuronium-bromide (0.06 mg/kg body weight; Mioblock; Sankyo, Tokyo, Japan) to steady the eyes (with positive pressure ventilation). After anesthesia was achieved, the dog was placed in the prone position. Prior to the placement of an eyelid speculum, the eye undergoing angiography was treated with drops of 0.4% oxybuprocaine hydrochloride (Benoxil ophthalmic solution; Santen, Osaka, Japan). The animal's head was positioned in a manner enabling the center of the camera field to focus easily on the fundus. Before angiography, we gave each dog an injection of metoclopramide (0.2 mg/kg/bw subcutaneously; Primperan; Astellas, Tokyo, Japan) and an intravenous injection of lactated Ringer's solution (10 ml/kg/bw/h IV; Solulact; Terumo, Tokyo, Japan) to prevent adverse reactions to the angiographic dye.

Indocyanine green angiography: ICG dye (Diagnogreen; Daiichi, Tokyo, Japan) was injected (1.0 mg/kg body weight) as a bolus through an intravenous catheter in the cephalic vein and followed by a 5-ml saline flush. The timer was started immediately after the saline flush, and sequential photographs (30 images per second) were recorded on videotape for approximately 90 min. From the onset of angiography the infrared light intensity of the fundus camera was set to approximately 20 mW/cm^2 (the highest setting), and the intensity was adjusted to match luminance if fluorescence increased excessively; this was a precaution for keeping the visualization clear on the angiograms. After completing angiography of each eye, we used the recorded videotapes for detailed observation and measurement of the ICG angiogram.

Fluorescein angiography: We injected FLUO dye (10 mg/kg/bw; Fluorescite 10%; Alcon, Tokyo, Japan) as a bolus, in the same manner as for ICG angiography and took angiograms according to the method of De Schaepdrijver *et al.* [4].

Calculations: The video timer automatically recorded the inflow times and filling times for the ten eyes examined, and from those recordings we calculated the means and standard deviation (SD) by Excel.

RESULTS

ICG angiography enabled clear visualization of the choroidal vasculature in all dogs, as shown by a representative angiogram in Fig. 1. After administration of the dye into the cephalic vein, inflow of the dye into the choroidal arteries began at approximately 8.4 ± 3.6 (mean ± SD) sec. The choroidal arteries, in spite of their narrow diameter, could be visualized distinctly as the arteries coursed radially from the periphery of the optic disc toward the equator of the eye (Fig. 1A). Inflow into the choroidal arteries coincided with that of the retinal arteries (8.5 ± 3.4 sec) in the peripapillary area.

In the choroidal veins, inflow of the ICG dye began at 10.2 ± 4.1 sec after administration, and many wide-diameter veins were seen running parallel to the choroidal arteries. All the choroidal veins and the retinal veins were filled within 17.5 ± 6.1 sec of administration of the dye, and fluorescence of the optic disc began to increase progressively. With full fluorescence of the veins throughout the entire fundus, fluorescence of the choroidal arteries decreased gradually. On filling to capacity, the choroidal veins, with their wide diameter made visible by fluorescence, could be easily detected as they coursed radially toward the tapetal and the non-tapetal fundus (Fig. 1B).

The ICG angiograms revealed innumerable fluorescence dots in the tapetal fundus that did not show up on the FLUO angiograms (Fig. 2). On the contrary, the capillaries, arterioles, and venules of the retina did not show up on the ICG angiograms (Fig. 2A) but were clearly visualized on the FLUO angiograms (Fig. 2B).

On the ICG angiograms, a diffuse fluorescence was seen over the entire ocular fundus, beginning at 13.2 ± 2.2 min after administration of the dye, and the choroidal vessels faded gradually from that time, as shown in Fig. 3. In spite
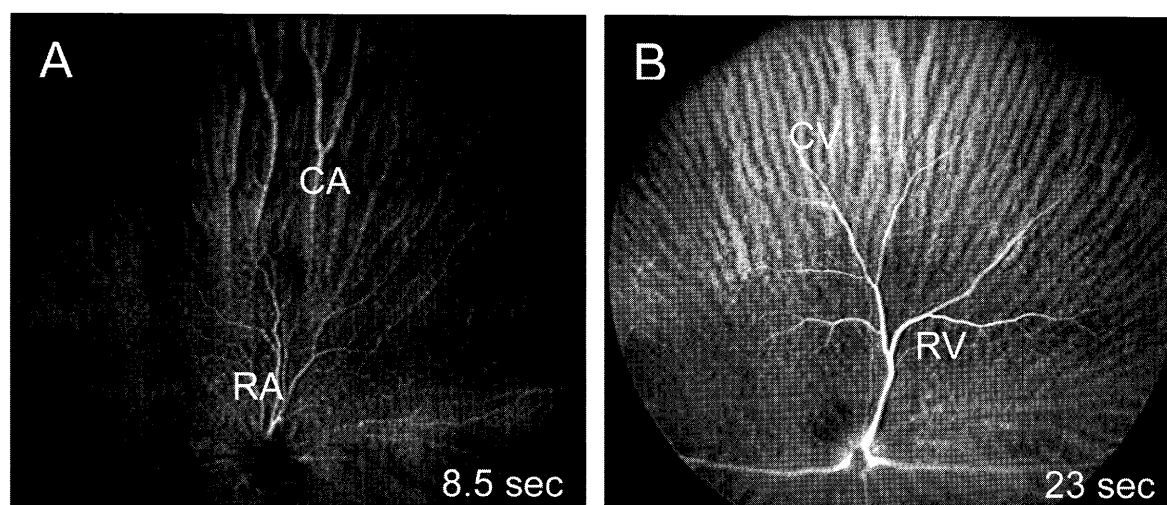
Fig. 1.   ICG angiograms showing ICG dye inflow into choroidal arteries and veins in the right eye of a healthy beagle dog, recorded at wide-angle. A: The choroidal arteries extend radially toward the equator. B: The choroidal veins, running beside the choroidal arteries, are the vessels having a wider diameter than the choroidal artery. CA, choroidal artery; CV, choroidal vein; RA, retinal artery; RV, retinal vein. Lower right: time lapse after intravenous administration of the dye into the cephalic vein.
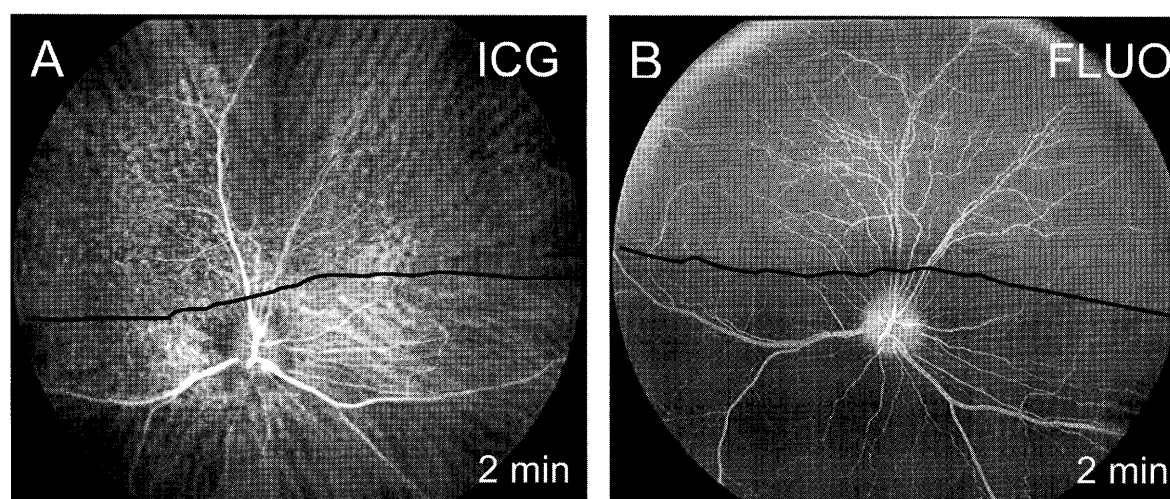

Fig. 2.   ICG and FLUO angiograms, right eye of a beagle dog, recorded at wide-angle. Filling characteristics of the two dyes can be compared in the choroidal vessels. A: ICG angiogram distinctly shows good filling of the choroidal vessels. The main retinal vessels are also visible, but resolution of the smaller retinal vessels is poor. B: FLUO angiogram shows the filling of all retinal vessels, but the choroidal vessels are not visible. In panel A, many fluorescence dots can be seen in the tapetal fundus (above the black line) but not in panel B. Black line divides tapetal fundus (above) from the non-tapetal fundus (below).

of vascular fading, the fluorescence provided by ICG permitted sharp examination of the vasculature up to fading of the choroidal vessels and optic disc at 58.3 ± 5.3 min after administration of the dye (Fig. 3), and diffuse fluorescence of the fundus itself continued throughout the 90-min examination period used to obtain each angiogram in the study.

In contrast to the 50-degree field of view, the wide-angle technique provided angiograms with an approximate 70-degree field of view.

DISCUSSION

The results of the present study support our hypothesis that indocyanine green (ICG) angiography employing the infrared-sensitive charged coupled device (CCD) would make it possible to assess the choroidal circulation in dogs, a necessary step in diagnosing choroidal diseases. On the ICG angiograms, the choroidal vessels could be visualized sharply, whereas on the FLUO angiograms the vessels of the retina were visible but not those of the choroid.

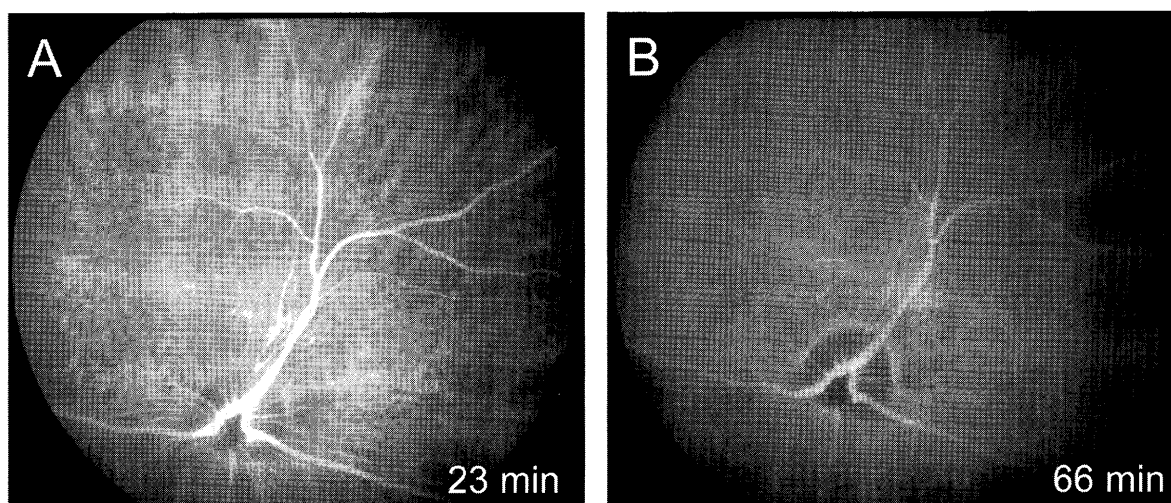Progression of the ICG dye through the choroidal circula-

Fig. 3.   Fading of an ICG angiogram, right eye of a beagle dog, recorded at a 50° angle for narrowing in on the fading parts. A: Diffuse fluorescence is seen throughout the ocular fundus. B: Although the choroidal vessels and the optic disc have almost faded, diffuse fluorescence continued to the end of our 90-min examination. Lower right: time lapse after intravenous administration of the dye into the cephalic vein.

tion resembled that reported in human ophthalmologic angiography [12, 15]. Beginning with inflow of the dye into the choroidal vessels of the dog and ending with fading of the vessels, the process can be classified into three phases (early, middle, and late), similar to those established by Iida and by Orlock in human angiography [12, 15]. The early phase, namely, that of choroidal arteriovenous inflow, was found to continue in dogs for approximately 13 min after administration of the dye. Within that period, three subdivisions mimicking the human choroidal vasculature are also evident: first, onset of inflow into the arteries; next, onset of inflow into the veins; and finally, the venous period exhibiting a brighter fluorescence of the veins than of the arteries. These sub-periods are potentially useful to veterinary ophthalmologists in discerning abnormal inflow and abnormal filling in individual choroidal vessels, thus enabling recognition of early signs of ischemia, vascular occlusion, pulsating vessel or other pathologic disturbances [10].

After the early phase, namely, inflow of the dye throughout the choroidal arterial-venous circulation, progression of the dye in the middle phase in dogs is characterized by gradual fading of the choroidal vessels concomitant with overall diffuse fluorescence lasting approximately 13- to -60 min after the dye is administered. This middle phase makes it possible to recognize active neovascularization and also atrophy in the choroid [10].

The late phase of progression of the dye in its sojourn through the dog choroid is characterized by prominent diffuse fluorescence of the choriocapillaris in the entire fundus from approximately 60 min after the dye is administered. This late phase allows us to recognize atrophy of the choriocapillaris and leakage of abnormal choroidal vessels [6]. This three-phase classification of the coursing of the ICG dye in the choroid layer offers potential for evaluating the

status of the choroid in isolation and in determining the optimum timing for obtaining ocular angiograms in dogs.

Although we found that progression of ICG dye in the dog choroid coincides with progression of the dye in the human choroid, four findings in the present study do not agree with results described in human studies. First, inflow time in the choroidal and retinal arteries; second, the point of origin of the choroidal arteries; third, dots on the fundus; and fourth, onset of the late phase of the dye progression.

First, in our study, inflow of the dye into the choroidal arteries (8.4 ± 3.6 sec) and retinal arteries (8.5 ± 3.4 sec) occurred almost simultaneously in the dogs, whereas in the human the dye inflow into the retinal arteries lags behind that of the choroidal arteries by approximately 1 sec [2, 24]. This timing variation may be explained by the architectural difference between the dog vasculature and the human vasculature. In the human eye, the central artery supplies the retinal arteries, and the ciliary arteries supply the choroidal arteries [2]. In contrast, the dog has no central artery but has only the ciliary arteries [17], which supply both the retinal and choroid arteries; consequently, the inflow of the choroidal arteries coincides with that of the retinal arteries in dogs but not in humans.

Second, we found that the choroidal arteries originate predominantly around the optic disc in the dog, but these arteries originate in the macula of the temporal ocular fundus in humans [24]. Although in subhuman primates the blood flow through the choroid, calculated as flow per area, is almost 10 times as high in the submacular area as in the periphery [5], in cats no such regional difference has been reported [14]. As the ocular fundus of cats closely resembles that of dogs and lacks a true macula, presumably the optic disc would be the normal origin of the choroidal arteries in dogs because dogs also lack a true macula.

Third, as for the many fluorescence dots observed on the tapetal fundus, we believe that what appears as dots are, in fact, the vessels running vertically within solely the tapetal layer, which does not exist in the human eye [17].

Finally, onset of the late phase in our dogs was approximately double the time required in humans, which is reported to occur 20- to -30 min after injection of the dye [18]. We attribute this difference to the species-specific half-life of ICG. The half-life of ICG in dogs (9 min) [3] is slightly more than double the half-life of the dye in humans (3–4 min) [9]. On the other hand, some unknown effect from anesthesia cannot be ruled out, since anesthesia was used in the present dogs but is not commonly used in ocular angiography in humans. Although deviating from descriptions of the human choroid, these four variations noted in our study appear to be normal in dogs, particularly since the same peculiarities were seen in all eight dogs in the study. Further studies are needed to clarify the normal from the abnormal.

Our low dose (1.0 mg/kg body weight) of ICG provided fluorescence at sufficient intensity and duration for us to examine the vasculature of the choroid as well as the entire fundus, including the tapetal area unique to veterinary medicine. In human trials, Guyer *et al.* administered ICG at 1.0–2.0 mg/kg body weight [8]; in rabbits, Kano *et al.* used 0.8–1.6 mg/kg/bw [13]; and in monkeys Suzuki used 1.0 mg/kg/bw [21].

The wide-angle technique, used here for the first time in dogs, provided far more detailed and useful angiograms than the 50-degree angle. The approximate 70-degree field of view permitted us to evaluate the choroidal arteries and veins separately, trace the start-to-finish course of the choroidal vessels, and record the entire tapetal fundus on a single photograph. These advantages attest to the important possibilities offered by the wide-angle technique for application to ICG angiography in dogs.

We propose the complementary use of ICG and FLUO angiography in dogs, as is customary for humans. ICG angiography enables visualization of the choroidal vessels, while FLUO angiography enables visualization of the retinal vessels, as borne out by this study. While either angiography approach provides unique information, a combination of the two methods may be necessary for diagnosing the wide spectrum of diseases of the ocular fundus, where pathological disorders may occur not just in the retina or the choroid but possibly in the circulation of both the layers [2]. Research is in progress to clarify the extent to which the results of this study can be applied to the various chorioretinal diseases in dogs.

In conclusion, the present study provides new and important information on the possibilities afforded by ICG angiography as a diagnostic aid in evaluating the choroid in dogs. The infrared-sensitive CCD fundus camera enables high-resolution ICG angiograms to be obtained. As borne out by this study, ICG angiography is accurate and reliable, and it is simple enough for clinical application. Used in combination with FLUO angiography, ICG angiography is potentially useful in planning early treatment and in determining the prognosis of chorioretinal diseases and maintaining long-term health of the eye in dogs.

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