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(54) **METHODS FOR DIAGNOSING AND TREATING CONDITIONS ASSOCIATED WITH ABNORMAL VASCULATURE USING FLUORESCENT DYE ANGIOGRAPHY AND DYE-ENHANCED PHOTOCOAGULATION**

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(57) **ABSTRACT**

Methods concerning medical uses for fluorescent dyes, e.g., Indocyanine green (ICG), fluorescein, rose bengal, for diagnosis and treatment. Methods for enhancing the clarity of fluorescent dye angiograms using relatively high dye concentrations, methods for determining the direction of blood flow within a blood vessel using fluorescent dye angiograms, and methods of identifying blood vessels that feed a lesion, such as a CNV or tumor. Methods of reducing the flow of blood into lesions incorporating dye-enhanced photocoagulation are also provided.

83 Claims, No Drawings

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METHODS FOR DIAGNOSING AND TREATING CONDITIONS ASSOCIATED WITH ABNORMAL VASCULATURE USING FLUORESCENT DYE ANGIOGRAPHY AND DYE-ENHANCED PHOTOCOAGULATION

FIELD OF THE INVENTION

The present invention relates generally to methods for diagnosing and treating conditions associated with abnormal vasculature.

BACKGROUND OF THE INVENTION

Fluorescent dyes, such as indocyanine green (ICG), have been used for years in connection with angiography to diagnose and treat vascular abnormalities that occur in the eye, e.g., choroidal neovascularization (CNV). CNV is a cause of Age-Related Macular Degeneration (ARMD), which is the leading cause of significant visual impairment in the elderly.

CNV originates in the choroidal blood vessels, the latter lying adjacent the retina of the eye. When CNV forms, it may intrude into and displace a portion of the sensory retina from its normal position, thereby distorting vision. Vision may also be blocked entirely if hemorrhage of the CNV occurs.

One method of diagnosing and treating ARMD is by laser photocoagulation of the CNV. This treatment, however, is successful to the extent that the CNV can be accurately mapped. This is because the CNV is, by definition, in the macular area and often encroaches on the fovea. Application of photocoagulation close to the fovea can result in the destruction of high acuity vision and/or accelerated growth of the CNV.

Generally, mapping of CNV is completed using angiograms. Angiograms are images of blood vessels, obtained by injecting a fluorescent dye into the blood stream prior to obtaining an image. As any of several dyes may be used, and because each dye fluoresces at its own particular wavelength, a radiation source that emits light (radiation) at that particular wavelength (e.g., a low-powered laser provided using fiber optic cables incorporated into a fundus camera) is used to illuminate the eye. Such a light source is part of a fundus camera, which also includes a CCD video camera. At or about the time of dye injection into the animal, the fundus camera begins capturing images, i.e., angiograms, of the eye at specific time intervals. The angiograms provide a record of the extent of dye movement within the ocular vasculature at each specific time interval.

More specifically, after the dye is injected into the body, the dye enters the vasculature of the eye and begins to fluoresce due to the presence of the appropriate excitation radiation (light). The fluorescing dye, being mixed with the ocular blood, provides each angiogram with an accurate illustration of the extent of ocular blood flow through the ocular vasculature at that moment. By comparing a series of angiograms of the same vasculature over a given time period, one is able to map the vasculature and determine the location of a CNV, and may then move to treat this abnormality, e.g., by laser photocoagulation of the CNV itself.

While the foregoing methodology has met with success, several issues remain. One is the clarity of the angiograms obtained using the previously described diagnostic methods. Clearly, any improvements in the angiogram clarity would result in a more accurate diagnosis, and, more significantly, allow a physician to more accurately locate a CNV requiring treatment.

Further, the medical uses of fluorescent dyes outside of the foregoing diagnosis and treatment procedures has been relatively limited. Other known uses for one such dye, ICG, are limited to diagnostic procedures, such as determining cardiac output, hepatic function and liver blood flow.

Accordingly, a need exists for methods of diagnosing and treating ocular vascular abnormalities, e.g., CNV, that overcome the aforementioned problems inherent in known methods of fluorescent dye angiography and photocoagulation. Further, and in view of the successful use of fluorescent dyes as diagnostics for certain limited conditions, i.e., ophthalmic angiograms, hepatic function and liver blood flow and cardiac output, there remain questions as to whether the use of these dyes can successfully be expanded into the diagnosis and/or treatment of other conditions and disorders.

SUMMARY OF THE INVENTION

The present invention meets the foregoing and other needs in a variety of ways. In a first aspect, the present invention provides a method for enhancing the clarity of fluorescent dye angiograms using relatively high dye concentrations, leading to more accurate targeting of vessels during treatment. In a second aspect, the present invention provides a method that allows blood vessels feeding various types of abnormalities to be more readily identified, and thereafter treated. Several other aspects of the present invention provide new methods of diagnosis and treating abnormalities and conditions using fluorescent dyes. All of the inventive aspects may be used on animals, e.g., humans, dogs, cats, but are preferably used in connection with the diagnosis and treatment of human subjects.

In particular, the present invention is able to provide angiograms of enhanced clarity by administering a plurality of relatively small boluses at relatively high dye concentrations to an animal undergoing an angiographic procedure. In particular, the method includes introducing boluses of about 0.1 ml to about 1.0 ml of a liquid composition at spaced time intervals into the animal to at least partially fill the blood vessels with the composition, wherein the liquid composition comprises a relatively high fluorescent dye and a carrier. For example, when using ICG, the dye concentration would be at least about 30 mg/ml, preferably at least about 40 mg/ml and most preferably at least about 50 mg/ml. Light energy of a type and in an amount sufficient to cause the dye in each bolus to fluoresce as the dye flows through the blood vessels is then applied, and angiographic images obtained.

Another aspect of the present invention provides a method for determining the direction of blood flow within a vessel. This may allow a physician to more readily determine whether a particular vessel is feeding an abnormality, indicating that it should be treated. The method includes at least the steps of administering a liquid composition comprising a fluorescent dye and a carrier into the animal to at least partially fill the blood vessel with the composition. Energy of a type and in an amount sufficient to cause the dye in the blood vessel to fluoresce is then applied. Subsequently, energy of a type and in an amount in excess of that required to cause the dye to fluoresce is applied to a portion of the fluorescing dye passing through the blood vessel to cause that portion of the fluorescing dye to stop fluorescing. A series of angiographs of both the fluorescing dye, and of the subsequent non-fluorescing portion thereof (also referred to as the "bleached" dye portion), are obtained, and those angiograms are compared to determine the direction of relative movement of the bleached dye. The direction of relative movement of the bleached dye portion indicates the direction of relative movement of the blood flow in the blood vessel.

Other aspects of the present invention involve new indications for fluorescent dyes. For example, one indication permits a physician to locate a tumor in or adjacent to the wall of a body cavity of an animal. This method includes administering a liquid composition comprising a fluorescent dye and a carrier into the animal to at least partially fill the blood vessels of the body cavity with the composition; applying energy of a type and in an amount sufficient to cause the dye to fluoresce as the dye flows through the blood vessels of the body c obtaining at least one angiographic image of the fluorescing dye as the dye flows through the blood vessels of the body cavity; and analyzing the angiographic image obtained in the prior step to determine whether a tumor is present in or adjacent to the wall of the body cavity. Related methods for diagnosing other types of lesions, e.g., ruptured blood vessels, abnormal vasculature, are also provided.

In other important aspects, the present invention provides methods for treating the aforementioned conditions. One exemplary method reduces the blood flow through a vessel that carries blood into a tumor of an animal. This method comprises administering a liquid composition comprising a fluorescent dye and a carrier into the animal to at least partially fill a blood vessel that carries blood into a tumor with the composition, and applying energy to the blood vessel of a type and in an amount sufficient to excite the dye in the blood vessel, thereby increasing the temperature of any liquid adjacent the dye, the increase in temperature causing the blood within the vessel to coagulate relatively quickly, thereby reducing (and preferably halting completely) the rate of blood flow through that vessel into the tumor.

Other related aspects of the present invention include methods for reducing or eliminating tumors. These methods are preferably used after the tumors have been located using fluorescent dye angiography, the latter providing a means for precisely locating a tumor in a subject. Once the precise location of a tumor is determined, methods including dye-enhanced photocoagulation, direct injection of chemotherapeutic and/or anti-angiogenesis agents into the tumor, conventional application of radiation, and surgical removal of the tumor, are expected to be effective against the tumor when used either alone or in combination. These methods have the advantage of lessening patient trauma because the treatment can be closely focused on the tumor alone as opposed to the tumor and other healthy body tissue, and may be used in combination in a single treatment session. For example, a single session can include dye-enhanced photocoagulation of those vessels feeding blood into the tumor using an endoscope, followed by injection of chemotherapeutic and anti-angiogenesis agents via the endoscope directly into the tumor itself (as opposed to conventional IV administration).

The various aspects of the present invention will be more clearly understood upon reference to the following preferred embodiments.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Turning initially to the issues associated with angiogram clarity, a first aspect of the present invention provides a method for enhancing the resolution of angiograms. This enhancement is provided by the introduction of a plurality of relatively small, yet highly dye-concentrated, boluses of a fluorescent dye composition into an animal, and subsequently obtaining angiograms as the composition passes through the vasculature of interest. The use of this method provides for a greater degree of fluorescence in the composition, and hence greater resolution in the associated angiogram, as compared to angiograms obtained using a composition having a conventional dye concentration.

Prior to the discovery of the present invention, there was no recognized need in any diagnostic or therapeutic procedure for using a fluorescent dye at a relatively high concentration. For example, one example of a suitable dye, ICG, has been marketed for years for use in angiography. The present package insert for IC-GREEN™ (ICG, manufactured by Akorn, Inc., Decatur, Ill.) suggests an optimal concentration of 20 mg ICG/ml for angiography (at 2 ml, providing a total ICG dose of 40 mg), depending upon the imaging equipment and technique used.

In contrast, this aspect of the invention includes introducing boluses of a liquid composition comprising a fluorescent dye at a concentration that is higher than that previously used. This concentration should be at least about 1.5 times (e.g., about 30 mg/ml for ICG), preferably at least about 2 times (e.g., about 40 mg/ml for ICG) and most preferably about 2.5 times (e.g., at least about 50 mg/ml for ICG) the highest known angiographic diagnostic concentration. The boluses are advantageously small in volume, about 0.1 ml to about 1.0 ml, and may be of the same or different volume. The boluses are introduced at spaced time intervals into an animal to at least partially fill the blood vessels of interest with the composition. After this administration, light energy of a type and in an amount sufficient to cause the dye to fluoresce as the dye flows through the blood vessels is applied, in accordance with procedures known in the art, and angiographic images are obtained. The images obtained provide higher levels of resolution than those obtained using conventional dye (e.g., ICG) compositions.

While not being bound to any particular theory, it is believed that the enhancement of resolution is due to the greater number of dye molecules present in a given wave front transiting a blood vessel, and a recognition that CCD cameras (typically used to obtain angiographic images) generate relatively high signal-to-noise ratios. With the relatively greater number of dye molecules being present in a particular dye "wave front," a greater the number of photons are generated by the dye upon exposure to radiation, providing better image quality even when the relatively high signal-to-noise ratio CCD cameras are used.

The total quantity of the liquid composition administered through a plurality of boluses (or as a single bolus, if desired) should be sufficient to permit readable angiographic images to be obtained and analyzed when using a CCD camera. This quantity may equal that administered using conventional formulations, but is advantageously greater, e.g., at least about 1.5 times the amount of dye administered using conventional formulations. More advantageously, at least twice that amount, preferably at least three times that amount, and most preferably, at least five times the amount of conventional formulations is administered. Optionally, after the administration of each bolus, a saline flush can be administered to aid the circulation of the liquid composition throughout the blood vessels of interest.

The dyes useful in the present invention should be able to fluoresce in the presence of radiation of a certain wavelength, and to permit angiographic images of blood vessels of higher quality to be obtained as compared to angiograms obtained using conventional dye concentrations. Preferably, the dyes should also be able to generate thermal energy when exposed to radiation. The dyes should therefore

be selected to at least permit diagnostic procedures, while preferred dyes function for both diagnostic and treatment procedures.

Treatment methods using dye-enhanced photocoagulation discussed herein comprise applying radiation of a certain wavelength (based upon the dye used) on a portion of an undesirable dye-carrying blood vessel. The radiation wavelength is selected to "excite" the dye; the absorption of such radiation by the dye causes the temperature of the dye to increase. As the correlation between radiation wavelength and increase in dye temperature is well known to those skilled in the art, this data will not be repeated herein. As the dye temperature increases, the temperature of the surrounding blood and vessel tissue increase. This increase in temperature hastens the rate at which blood clots in and adjacent that portion of the vessel onto which the radiation is applied. This clotting, in turn, leads to partial, or preferably complete, obstruction of the vessel in or adjacent the portion of the vessel onto which the radiation was applied.

The dye-containing composition used in this and the other treatment methods disclosed herein may vary widely. One limit on the dye concentration is that sufficient dye should be present in composition, and more importantly the targeted vessel, to permit at least partial obstruction of the target vessel by the dye-enhanced photocoagulation methods discussed herein. Further, the novel diagnostic methods disclosed in the following paragraphs may also use a wide range of dye concentrations, with the limitation that sufficient dye should be present in the composition (and targeted vessels) to permit the angiograms taken in conjunction with those methods to be analyzed.

One method of determining the degree of vessel obstruction is by analyzing angiograms taken after treatment is completed, and after the dye has left the treated vessel. For example, if the treatment results in total obstruction of a CNV feeder vessel, an angiogram of the downstream portion of the vessel, e.g., the CNV itself, will not reveal any dye fluorescence. Partial obstruction should reveal a lower degree of fluorescence.

A number of fluorescent dyes are known that are acceptable for use in the composition of the various inventive methods described herein. Exemplary dyes include fluorescein, rose bengal, ICG and analogue members of the tricarbocyanine dyes, and any other dye which meets the criteria described herein for diagnosis and/or treatment procedures. The preferred fluorescent dye is ICG because it is readily available, has long been approved for administration to humans for ophthalmic angiography and other unrelated indications, and is suitable for both diagnosis and treatment procedures. As the peak absorption and emission of ICG lies in the range of 800–850 nm, a light source emitting such wavelengths should be used when obtaining angiographic images during diagnosis, as well as during any subsequent treatment procedure.

The dye compositions may further include a pharmaceutically-acceptable carrier. The carrier enhances the administration of the fluorescent dye to a patient, the latter being either intravenously or by other suitable means. The choice of carrier will be determine in part by the particular fluorescent dye used, as well as by the particular route of administration of the liquid composition. The carrier should be compatible with both the fluorescent dye and the tissues and organs of the subject that come into contact with the liquid composition. Moreover, the carrier should not interfere with the energy applied or angiographic images obtained following administration.

Illustrative of suitable carriers include water, saline, alcohols, red blood cells (RBC), glycerin, polyethylene glycol, propylene glycol, polysorbate 80, Tweens, liposomes, amino acids, lecithin, dodecyl sulfate, lauryl sulfate, phospholipid, Cremophor, desoxycholate, soybean oil, vegetable oil, safflower oil, sesame oil, peanut oil, cottonseed oil, sorbitol, acacia, aluminum monstearate, polyoxyethylated fatty acids, povidone and mixtures thereof. Advantageously, the carrier is water. Preferably, however, the composition will include components that increase the degree of dye fluorescence, e.g., alcohols such as ethanol and surfactants such as the Tweens. Optional components that may be present in the composition include tonicity and/or pH adjusters, e.g., NaOH, HCl, tribuffer phosphate, tris buffer and the like. In addition, the composition may include thrombin or other known blood clotting compounds that would function to further enhance blood clotting during and after treatment.

The fluorescent dye composition may initially be provided as a lyophilizate for reconstitution before use, or as a pre-mix, in a vial or syringe.

As mentioned above, and in a related aspect of the present invention, RBCs may be used as a carrier for the fluorescent dye. This technique is referred to herein as RBC doping. The RBC as a carrier has advantages in that it is a normal constituent of circulating blood and, despite the relative large volume (and hence large dye-carrying capacity) of each RBC, RBCs can nevertheless readily move throughout the circulatory system—deforming to enable movement through even the small diameter capillaries. Further, and while not desiring to be bound to any particular theory, the use of doped RBCs provides additional advantages pertaining to clot formation. In particular, the size of clot formed during the treatment methods described herein depends upon the amount of dye present at the vessel treatment site, the amount of radiation energy delivered thereto and the distribution of the dye molecules associated with the RBCs. The greater the number of dye molecules associated with the RBCs, the more sizable the clot will be when exposed to appropriate radiation during the treatment phase. Of course, if the clot is large enough, vessel closure will be permanent. However, if smaller, as is often the case using conventional treatment methods, the clot will resolve, requiring additional treatment. The doping of dye in RBCs reduces the variability in clot formation because it increases the fraction of dye molecules associated with RBCs at the treatment site, thereby increasing the probability that a sizable clot is formed during treatment.

The object of the procedure is to remove the content of the RBCs, and then refill the RBCs with hemoglobin and dye, e.g., ICG, and, if desired, other clot potentiating compounds, e.g., fibrin. When the use of RBC doping is indicated, the following exemplary procedure may be followed to provide the doped composition for use in the various inventive methods described herein. Preferably, a small amount of the subject's blood is withdrawn (about 10–15 ml), although any compatible blood may be used, and is centrifuged to permit removal of the serum. The remaining RBCs are washed in normal PBS to remove proteins from the RBC surface. The washed RBCs are placed in a cooled hemolizing solution, and incubated therein for about 5 min. The pH of the solution is readjusted to 7.2, and ICG is added. The solution is again incubated at 37° C. for about 45–60 min. If desired, other compounds that assist in clotting, e.g., fibrin, may be added at this stage. The solution is then centrifuged at about 500 g for about **6** min, and the supernatant is removed. The resulting cells are washed several times to

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