(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) **Title:** METHOD OF ENDOVASCULAR BRAIN MAPPING

(57) **Abstract:** Disclosed are the following strategies for endovascularly mapping the brain with a chemical agent capable of staining the preselected region of the brain to a color visibly contrasting with non-stained portions of the brain and of passing through the blood-brain barrier to the preselected region of the brain: 1) Passive transport: (a) pro-drug, (b) Modification of mapping agent to mimic molecules that readily cross the BBB (e.g., amino acid, glucose, etc.); 2) Active transport; 3) Receptor-mediated transport (e.g., antibody mediated); 4) Blood brain barrier (BBB) manipulation; 5) Emulsification of agent (such as β-carotene, organic dye, etc.) to stain capillary endothelium; 6) Embolization of brain capillaries; 7) Grafted-nanoparticle systems for brain delivery of mapping agent.

Method of Endovascular Brain Mapping

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to a novel brain mapping system especially adapted for

the enhancement of Craniotomy Guidance.

Description of the Prior Art:

Brain mapping is the attempt to specify in as much detail as possible the localization

of function in the human brain (Savoy RL: History and future directions of human brain

mapping and functional neuroimaging. Acta sychologica 07:9-42, 2001). A variety of brain-

mapping techniques exist, ranging from the historic lesion studies to functional magnetic

resonance imaging (fMRI) (Llinás, RR: Mapping Brain Terrain. Neurobiology of Disease 7

499-5 00, 2000; Orison WW, Lewine JP, Sanders JA, Hartshorne MF: Functional Brain

Imaging. St. Louis: Mosby, 1995.

The oldest and simplest form of brain mapping is lesion studies (Corkin 5, Amaral

DG, Johnson KA, Hyman BT: H.M's medial temporal lobe lesion: findings from MRI.

Journal of Neuroscience 17:3964-3979, 1997; Kolb B, Whishaw IQ: Fundamentals of human

neuropsychology (4th ed.). New York: Freeman, 1996; Tootell RBH, Iale AM, Sereno MI,

Malach R: New images from human visual cortex. Trends in Neuroscience 19(11):481-489,

1996). The anatomic basis of neurological function has been inferred from the correlation of

clinical findings with anatomical studies, whether by autopsy or radiology, in patients with

known discrete brain lesions. This is the method by which the knowledge of localization was

long advanced, but with marked limitations. Lesions are not controlled and often involve

multiple areas with multiple functions. Gray matter subserving one function often overlies

white matter carrying fibers subserving an unrelated function. Nevertheless, our basic

understanding of neuroanatomy is largely based on these historic studies, and forms the basis

for the crude form of preoperative brain mapping currently employed for many neurosurgical

procedures the expectation of the neurosurgeon that a given resection has a certain

probability of producing a given neurologic deficit.

Starting with Penfield and his associates, the clinical utility of brain mapping for

operative planning led to the practice of intraoperative stimulation in awake patients

(Penfield, supra; Penfield W, Jasper H: Epilepsy and the functional anatomy of the human

brain. Boston, MA: Little, Brown and Company, 1954). This was most useful for mapping

the motor or language cortex, but found application in other areas as well. These studies, in

addition to the lesion studies discussed above, provided much of the current understanding of

basic neuroanatomy. The disadvantages of this approach, however, are clear. The increased

operative time and associated risk of infection, the broad cortical exposure required, and the

inconvenience of completely awakening the patient during open brain surgery while

adequately controlling pain all limit the use of these techniques. The process is also

painstakingly slow, testing point by point accompanied by questioning of examination of the

patient. Additionally, the functions tested are limited to those that can be tested at the bedside

in a patient that is physically unable to move the head. Obviously, gait functions or postural

stability could not be assessed in this way. Similarly, detailed neuropsychological testing is

generally impractical in such a setting.

A more modern development of this technique, primarily employed in preoperative

planning of epilepsy surgery, is the use of intracortical depth electrodes and arrays of

subdural electrodes that are surgically implanted and then used to collect data passively for a

period of time before the definitive neurosurgical procedure (Puce A, Constable RT, Luby

ML, McCarthy G, Nobre AC, Spencer DD, Gore JC, Allison T: Functional magnetic

resonance imaging of sensory and motor cortex: comparison with electrophysiological

localization. Journal of Neurosurgery 832(2):262-270, 1995.). Similarly, many neurosurgeons

use microelectrode recording and stimulation to precisely localize deep nuclei (Halgren E,

Walter RD, Cherlow DG, Crandall PH: Mental phenomena evoked by electrical stimulation

of the human hippocampal formation and amygdala. Brain 101:83-1 17, 1978; Vignal JP,

Chauvel P, Halgren E: Localized face processing by the human prefrontal cortex: imulation-

evoked hallucinations of faces. Cognitive Neuropsychology, 17:281-291, 2000.). The obvious

disadvantage of these approaches is their invasive nature that requires more than one major

open brain operation. This includes the accompanying risks of hemorrhage and neurological

deficit secondary to direct tissue trauma.

Two modern techniques offer the ability to perform similar tests non-invasively by

using electromagnetic fields to induce changes within the cortex that stimulate the tissue or

that behave as a temporary lesion. In transcranial electrical stimulation (TES), electrodes are

affixed to the scalp and current is passed between them to stimulate underlying cortex. As the

procedure is uncomfortable, this technique is seldom used. Transcranial magnetic stimulation

(TMS) typically causes no direct unpleasant effects other than noise, and is thus more

frequently employed. In TMS, large coils are used to generate rapidly changing magnetic

fields within the brain, inducing localized currents that can either stimulate the cortex in that

location or temporarily inactivate that cortex (George MS, Lisanby SH, Sackeim HA:

Transcranial magnetic stimulation: applications in neuropsychiatry. Archives of General

Psychiatry 56:300-311, 199; Pascual-Leone A, Bartres-Faz D, Keenan JP: Transcranial

magnetic stimulation: studying the brain-behavior relationship by induction of 'virtual

lesions', Philosophical Transactions of the Royal Society of London B 354:1229-1238, 1999;

Pascual-Leone A, Meador KJ: Is transcranial magnetic stimulation coming of age? Journal of

Clinical Neurophysiology 15(4):285-287, 1998; Pascual-Leone A, Walsh V, Rothwell J:

Transcranial magnetic stimulation in cognitive neuroscience-virtual lesion, chronometry, and

functional connectivity, Current Opinion in Neurobiology 10:232-237, 2000; Stewart L,

Ellison A, Walsh V, Cowey A: The role of Transcranial Magnetic Stimulation (TMS) in

studies of vision, attention and cognition. Acta Psychologica 107:275-291, 2001; Walsh V,

Cowey A: Magnetic stimulation studies of visual cognition. Trends in Cognitive

Neuroscience 2(3):103-1 10, 1998). The limitations of this technique are its very limited

spatial resolution, imprecise location, and the tendency of repetitive stimulation to induce

seizures.

Passive transcranial modalities have been employed as well. The most widespread is

electroencephalography (EEG), more commonly used for the evaluation of seizure disorders

(Gevins A: Electrophysiological imaging of brain function, in Toga AW, Mazziotta JC

(Eds.): Brain mapping: the methods. San Diego: Academic Press, 1996, 259-276; Lewine JD,

Orrison WWJ: Clinical electroencephalography and event-related potentials, in Ctrrison

WWJ, Lewine JD, Sanders JA, Hartshorne MF (eds): Functional brain imaging 327-368.

Boston, MA: Mosby-Year Book, 1995). It shares with its magnetic counterpart,

magnetoencephalography (MEG) (Lewine JD, Orrison WWJ: Magnetoencephalography and

magnetic source imaging, in Orison WWJ, Lewine JD, Sanders JA, Hartshorne MF (Eds.):

Functional brain imaging. Boston: Mosby-Year Book, 1995, 369-4 18; Lounasmaa OV,

Hämäläinen M, Han R, Salmelin R: Information processing in the human brain:

magnetoencephalographic approach. Proceedings of the National Academy of Science, USA

93:8809-8815, 1996), good the benefit of excellent temporal resolution, but is severely

limited in its application to brain mapping by very poor spatial resolution.

Finally, the autoregulation of the brain in increasing local blood flow according to

local metabolic activity (neuronal firing) allows for several brain mapping techniques

(Raichle ME: Behind the scenes in functional brains imaging: a historical and physiological

perspective. Proceedings of the National Academy of Sciences, USA, 95:765-772, 1998.),

including most forms of functional Magnetic Resonance Imaging (fMRI), positron emission

tomography (PET) scanning, or even optical measurement. These are very powerful

techniques, but suffer from the same weakness that all noninvasive strategies do when it

comes to preoperative planning, namely, inadequate spatial resolution correlated to the view

of the surgeon at craniotomy. Also, while they accurately identify the tissue that is active

during any given task, it is difficult without a lesion type study to discern incidentally active

tissue from tissue indispensable for neurologic functioning. Generally, the question answered

is "What tissue is active during task A?" rather than the question more important to the

surgeon, "What are all of the functions that will be impaired by the loss of this tissue, and to

what degree?"

Each of these known techniques has various strengths and weaknesses that are useful

for certain specific aspects of research in cognitive neuroscience. However, the primary use

of brain mapping in neurosurgery is the determination of the presence or absence of function

in a potential site of brain resection. Regardless of the indication for neurosurgical resection,

it is always desirable to avoid neurological deficits, and so it is desirable to attempt to clarify

the function of the tissue before resection. Most existing brain mapping techniques have some

characteristics that are favorable for preoperative planning. However, none of the existing

brain mapping techniques allows precise, direct, real-time visualization of brain parenchyma

that can be resected with assurance of no new post-operative neurological deficit, i.e., "silent"

brain parenchyma.

The ideal clinical brain mapping technique, however, allows precise definition of the

postoperative deficits to be expected with removal of a given portion of brain tissue, coupled

with the ability to subsequently precisely identify that tissue at the time of craniotomy.

SUMMARY OF THE INVENTION

The present invention relates to a new form of preoperative clinical brain mapping

using an endovascular approach that will promote improved neurological outcomes after

brain resection. This procedure is not intended for experimental cognitive neuroscience,

where the ability to compare results across multiple subjects and serially in time is important.

Rather, this technique is especially designed for individualizing the unique brain function

map of each patient who requires brain resection to improve his/her neurosurgical outcome.

One embodiment of the invention relates to a method for diagnostically imaging a

preselected region in a warm-blooded animal having a brain situated inside a blood-brain

barrier having an ambient permeability level, the method comprising the steps:

1) endovascularly administering a first chemical agent to the animal at a location proximal to

the preselected region outside the blood-brain barrier, where the first chemical agent causes

local brain anesthesia to the portion of the brain which is distal to the position of the

microcatheter (targeted brain). This first chemical agent is usually a very short acting

barbiturate medication such as Sodium Methohexital (Brevital®), or amobarbital (Amytal®).

This constitutes a "Superselective distal Wada test" (van Emde Boas W: Juhn A. Wada and

the sodium amytal test in the first (and last?) 50 years *J Hist Neurosci* 1999 Dec; 8(3):286-

92).

2) Performing detailed neurological, cognitive, and possibly psychological bedside testing to

determine the presence or absence of any new deficit.

3) endovascularly administering a second chemical or physical agent through the same

microcatheter which has remained in the exact same position after administration of the first

chemical agent proximal to the preselected targeted brain tissue following the administration

of the first chemical agent, the second chemical or physical agent being capable of staining

the preselected region of the brain to a color visibly contrasting with non-stained portions of

the brain, possibly passing through the animal's blood-brain barrier to the preselected region

of the brain when the blood-brain barrier is at the elevated permeability level but not at the

ambient permeability level. Alternatively, the second chemical agent could be attached to the

blood-brain barrier at the level of the brain capillary endothelial cells, or other location within

the pre-selected targeted portion of brain that was previously tested with the selective distal

Wada test using the first chemical agent as described above.

A second embodiment of the invention concerns an improved method involving

surgery on the brain of a warm-blooded mammal, the improvement comprising diagnostically

imaging a preselected region in a warm-blooded animal having a brain situated inside a

blood-brain barrier having an ambient permeability level, the imaging comprising the steps:

endovascularly administering a first chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier, where the first chemical

agent is capable of increasing the permeability of the blood-brain barrier from the ambient

level to an elevated permeability level;

endovascularly administering a second chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier following the

administration of the first chemical agent, the second chemical agent being capable of

staining the preselected region of the brain to a color visibly contrasting with non-stained

portions of the brain and of passing through the animal's blood-brain barrier to the preselected

region of the brain when the blood-brain barrier is at the elevated permeability level but not at

the ambient permeability level.

Additional embodiments of the invention relate to the following strategies for

endovascularly mapping the brain: 1) Passive transport: (a) pro-drug, (b) Modification of

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mapping agent to mimic molecules that readily cross the BBB (e.g., amino acid, glucose, etc.); 2) Active transport; 3) Receptor-mediated transport (e.g., antibody mediated); 4) Blood brain barrier (BBB) manipulation; 5) Emulsification of agent (such as β-carotene, organic dye, etc.) to stain capillary endothelium; 6) Embolization of brain capillaries; 7) Grafted-nanoparticle systems for brain delivery of mapping agent.

Still further embodiments of the invention relate to kits for endovascularly mapping the brain and their use wherein the kits comprise, as a first component, a chemical agent being capable of staining a preselected region of the brain to a color visibly contrasting with non-stained portions of the brain and, as further components, agents for enhancing transport to the preselected regions of the brain of the chemical agent.

Other embodiments of the invention relate to articles of manufacture and their use wherein the comprises packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent is effective for the endovascular mapping of the brain, and wherein the packaging material comprises a label which indicates that the pharmaceutical agent can be used for the endovascular mapping of the brain.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 depicts a fluorescein mapped rat brain; (a) dorsal view and (b) coronal section.

Fig.2 depicts a FD & C green #3 mapped rat brain; (a) dorsal view, (b) ventral view and (c) coronal section.

Fig. 3 sets forth the chemical structures of Iohexol (Omnipaque™) (a), Methohexital Sodium; 2,4,6 (1H, 3H, 5H) Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)-, monosodium salt (Brevital®) (b), γ-cyclodextrin (c) and doxorubicin (d).

DETAILED DESCRIPTION OF THE INVENTION

The novel methods of the invention enable those skilled in the art, e.g., to safely define margins of resection at craniotomy, along with identifying any expected neurological deficits associated with the resection.

There currently exists no system or procedure for the endovascular mapping of the brain. The Wada test, which is performed by infusing a short acting barbiturate, Brevital® (Jones Pharma Inc., St. Louis, MO) or sodium amytal, into the internal carotid artery in an awake patient, followed by detailed bedside neurological and cognitive testing, is a routine endovascular procedure that is performed to determine localization of dominant brain function such as language prior to major epilepsy surgery. The test is well known to most interventional neuroradiologists and endovascular neurosurgeons.

Any transient neurological or cognitive deficits that are detected are attributable to the portion of brain parenchyma that was infused with the short-acting barbiturate. The temporary deficits which occur because of Brevital® resolve back to baseline within several minutes; whereas the temporary deficits secondary to sodium amytal may last significantly longer. Because of recent improvements in endovascular techniques and microcatheter technology, it is now routine to catheterize distal branches of the anterior, middle, and posterior cerebral arteries. It is therefore possible to perform a superselective Wada test with transcatheter infusion of Brevital® at distal selective targeted brain parenchyma. This type of superselective Wada testing is often performed prior to endovascular embolization of intracranial pial arteriovenous malformations. Detailed neurological and cognitive examination is performed before and after the transcatheter super-selective transient anesthesia, and this would definitively determine if the target brain parenchyma is "silent" or "eloquent". If the target brain was proven to be "silent", an appropriate mapping agent could then be infiltrated into the target brain, so that the "silent" brain is clearly visualized during a

subsequent elective craniotomy. The neurosurgeon would then be certain that no deficit

would occur after resection of any brain that was mapped as silent. This could be useful for

epilepsy surgery, malignant glioma surgery, arteriovenous malformation (AVM) surgery,

functional neurosurgical resections, and other possible applications.

With the advent of modern endovascular techniques and the explosion in catheter

technology, it is now relatively routine to catheterize and selectively inject 3rd and 4th order

intracranial vessels. It is possible, therefore, to perform superselective anesthesia

endovascularly, essentially inducing a transient reversible neurologic lesion in selected and

targetedsections in the brain. Since angiography is performed with the patient awake, the

patient may be examined for any new deficit while the anesthetic is in effect. This could be

extended beyond basic neurologic examination to include even detailed neuropsychological

testing. The clinical usefulness of this ability for planning surgical resections has to date been

limited by the difficulty of correlating the findings at endovascular testing with the view of

the surgeon at craniotomy.

The present invention enables the marking of the vascular territory being studied with

a visible color, thereby enabling the neurosurgeon to precisely identify the tested territory.

The surgeon would then be certain that no neurological deficit would result from of the

resection of brain that was so marked or "mapped." This would result in vast improvements

in epilepsy surgery, brain tumor surgery, AVM surgery, and the like.

Several dyes are available that are widely used in foods and medications that are

minimally toxic. Prior studies have shown that many dyes can cross the blood brain barrier

and stain the brain parenchyma after disruption of the blood brain barrier with an osmotic

agent (Robinson PJ, Rapoport SI: Size selectivity of blood-brain barrier permeability at

various times after osmotic opening. Am J Physiol 253:R459-R466, 1987; Suzuki M, Iwasaki

Y, Yamamoto T, Konno H, Kudo H: Sequelae of the osmotic blood-brain barrier opening in

rats. J Neurosurg 69:42 1-428, 1988; Ziylan YZ, Robinson PJ, Rapoport SI: Differential

Blood-Brain Barrier Permeabilities to [^{14}C]Sucrose and [^{3}H]Inulin after Osmotic Opening in

the Rat). Fluorescein is a dye with minimal toxicity widely used in clinical practice,

particularly in ophthalmological applications (Albert DM and Jakobiec FA: Principles and

Practice of Ophthalmology Clinical Practice. Philadelphia, PA: WB Saunders Co, 1994.

Fluorescein angiography is an established method of retinal evaluation. Accordingly, the first

experiments that led to the present invention explored the use of selective blood-brain barrier

disruption followed by fluorescein infusion.

The ideal endovascular brain mapping agent should possess all of the following

characteristics: 1) Must be nontoxic systemically; 2) Must be clearly visualized, either

directly or indirectly with the assistance of accessory devices (ultraviolet illumination,

fluorescent detection, etc.; 3) Must be radiographically opaque for fluoroscopic visualization;

4) Must be capable of either crossing the blood-brain barrier (BBB) either with or without

temporary BBB manipulation techniques, or attaching to the capillaries within the selected

targeted brain.

The seven major approaches for accomplishing the goal of preoperative endovascular

brain mapping include: 1) Passive transport: (a) pro-drug, (b) Modification of mapping agent

to mimic molecules that readily cross the BBB (e.g., amino acid, glucose, etc.); 2) Active

transport; 3) Receptor-mediated transport (e.g., antibody mediated); 4) Blood brain barrier

(BBB) manipulation; 5) Emulsification of agent (such as β-carotene, organic dye, etc.) to

stain capillary endothelium; 6) Embolization of brain capillaries; 7) Grafted-nanoparticle

systems for brain delivery of mapping agent.

Modifications to the Zeiss operating microscope to allow clear visualization of

fluorescein stained brain territories have been described (Kuroiwa T, Kajimoto Y, Ohta T:

Development of a Fluorescein operative microscope for use during malignant glioma surgery.

A technical note and preliminary report, Surg Neurol 50:41-9, 1998.). However, the advantage in the simplicity of a visible spectrum dye is obvious. Therefore, selective blood brain barrier disruption followed by infusion of an aqueous suspension of FD&C Green #3 was attempted.

Once a given distribution of tissue has been tested and chosen for removal, preoperative embolization of that territory would be helpful to reduce operative bleeding during resection. It was theorized that if an appropriately colored embolic agent were used, it could contribute to the marking of the territory intended for resection. It was therefore decided to test the injection of tantalum powder, a black embolic agent that is radiopaque.

The first step of the endovascular brain mapping procedure is to perform superselective microcatheterization of the target artery feeding the target brain parenchyma in an awake patient in the endovascular suite. Secondly, the target brain parenchyma is infused with methohexital sodium (Brevital®) and performance of detailed neurological examination. If this provocative endovascular testing shows no change in the neurological or cognitive function, then this distribution is defined as "silent" brain parenchyma and is suitable for mapping. An appropriate mapping agent is then infused into the exact same distribution that will visually stain the "silent" brain parenchyma so that when craniotomy is performed, a clear, real-time margin is visualized and will allow the neurosurgeon to resect only the portion that has been proven to be "silent". If critical neurological function is found in the target brain parenchyma, then the microcatheter must be moved to another adjacent territory. This procedure is repeated at any distribution in the brain that may require surgical resection at a later date. At the conclusion of the preoperative endovascular brain mapping procedure, all silent brain parenchyma in the region of the target parenchyma will be "mapped" or visually marked with clear margins, in conjunction with embolization, if necessary. The final step in the procedure is to perform a previously planned craniotomy and resection of the

target lesion. The resection will be guided by the clearly visible mapped silent brain.

Therefore, it should be absolutely certain that no neurological or cognitive deficit would

occur after the brain resection. This will eliminate the need for other more cumbersome,

tedious, painful, and less accurate functional brain mapping procedures such as awake

cortical stimulation, electrocorticography with prolonged inpatient monitoring, and

stereotactic image-guided surgery.

The feasibility of brain-mapping is illustrated by the following non-limiting examples

carried out in animal models. It will be understood by those skilled in the art that before any

of the possible mapping agents is attempted in a human, it must first be confirmed to show

good visual clarity, and sharp margins between the mapped brain and the non-mapped brain

in an animal model.

Generally, Sprague Dawley rats were anesthetized and vascular access obtained by

ventral cervical cutdown. A catheter was introduced into the common carotid artery proximal

to the ICA. All other branches were clamped temporarily with aneurysm clips, permitting

flow to the ICA alone. The blood brain barrier was disrupted with a solution of arabinose. An

aqueous solution of Fluorescite® (fluorescein-Alcon Lab) or FD&C Green #3 with

Omnipaque® (tantalum powder-Hycomed) contrast agent was infused. Tantalum powder was

also tested without blood brain barrier disruption. The brains were harvested for examination

of the quality of staining.

EXAMPLE 1

Female Sprague Dawley rats were anesthetized with a mixture of Ketamine and

Xylazine (87 mg/ml/13 mg/ml, 0.1 ml/100 g body weight). Using a carotid cutdown method,

the CCA was exposed and a catheter was introduced into the lumen proximal to the ICA. A

ligature secured the catheter within the vessel. The external carotid artery and occipital artery

were clamped, preserving flow to the ICA. We followed a previously described method for

osmotic disruption of the blood brain barrier using a hypertonic solution of arabinose (2.7g of

arabinose was dissolved in 10 ml of sterile water under gentle warming, and the solution was

filtered to remove any undissolved crystals. 3.6cc of this solution was infused over 30

seconds. After a delay of six minutes, we infused a 10% solution of fluorescein mixed with

an equal amount of Omnipaque ®(Hycomed) contrast agent under fluoroscopic guidance.

The animal was then euthanized and the brain harvested.

EXAMPLE 2

The same procedure was repeated in another rat with an aqueous suspension of FD &

C green #3 (Figure 1) mixed with Omnipaque®. To test the use of tantalum powder, the same

procedure described above was performed without disruption of the blood brain barrier. A

small amount of tantalum powder suspension was then injected under fluoroscopic

visualization until the right hemispheric distribution was opacified.

In both examples, after fluorescein infusion, staining was evident on the cortical

surface (Figure 2) and into the deep white matter (Figure 3). The normal brain tissue

autofluoresces slightly at this wavelength. Appropriate filtration on the observation lens can

further improve contrast. After infusion of FD & C Green #3, the anterior and middle

cerebral artery distributions were clearly stained both on the cortical surface (Figure 4) and

into the deep white matter (Figure 5), with sparing of the posterior cerebral artery distribution

over the inferior portion of the temporal lobe (Figure 6). Finally, the embolic agent, tantalum

powder, produced dark staining of the larger caliber arterial brain cells but was insufficient in

its penetration to smaller arterioles and capillaries to be useful as a standalone agent (Figure

7). While tantalum proved insufficient as a stand-alone agent, this is likely secondary to its

size (50-100 mm) and irregular shape (Figure 8). A more uniform, smaller agent such as

Histoacryl® (Braun) added to an oil soluble dye such as Sudan Black B® (Sigma) and

Ethiodol (for radioopacity) will obviate the need for disruption of the blood brain barrier, and

hasten the procedure.

The toxicity profile of fluorescein or FD & C Green #3 is not severe (Albert et al,

supra; Kuroiwa et al, supra; Richard G: Flourescien and ICG Angiography Textbook: An

Atlas. Thieme, NY, 1998.). Fluorescein is widely used in retinal angiograms, and Fast Green

FCF is broadly approved for food and drug use. There is little data on the parenteral use of

FD & C Green #3, although there is evidence for mutagenicity in all coal tar dyes

(Anonymous: Fast green FCF. IARC monographs on the evaluation of carcinogenic risk of

chemicals to man Jan:16187-97, 1978; Das SK, Gin AK: Chromosomal aberrations induced

by secondary and tertiary amine-containing dyes and in combination with nitrite in vivo in

mice. Cytobios 54(216):25-9, 1988; Gin AK, Mukherjee A: Sister chromatid exchange

induced by secondary and tertiary amine containing dyes and in combination with nitrite in

vivo in mice. Cancer-Lett 52(1):33-7, 1990; Hansen WH, Long EL, Davis KJ, Nelson AA,

Fitzhugh OG: Chronic toxicity of three food colorings: Guinea Green B, Light Green SF

Yellowish and Fast Green FCF in rats, dogs and mice. Food and cosmetics toxicology

4(4):389-410, 1966; Ishidate M, Sofuni T, Yoshikawa K, Hayashi M, Nohmi T, Sawada M,

Matsuoka A: Primary mutagenicity screening of food additives currently used in Japan. Food

and chemical toxicology 22(8):623-36, 1984; Roychoudhury A, Gin AK: Effects of certain

food dyes on chromosomes of Allium cepa. Mutat-Res 223(3):313-9, 1989). There is also

data to suggest induction of sarcomas after chronic subcutaneous injections of FD & C Green

#3 (Nelson AA, Hagan EC: Production of fibrosarcomas in rats at site of subcutaneous

injection of various food dyes. Federation Proceedings 12:397-398, 19531953). The clinical

impact of any toxicity is expected to be very minimal because of the single exposure, and the

ability under fluoroscopy to stop dye infusion once the angiogram shows full perfusion. Any

direct toxicity to the stained parenchyma is likely of little clinical significance, as that stain is only applied to tissue that is intended to be removed very shortly thereafter.

Both fluorescein and FD&C Green #3 resulted in good staining of a selective vascular distribution. Tantalum powder darkly marked the main arterial branches but did not show good parenchymal staining. Thus, the invention is highly valuable for the endovascular marking of tissue for clear identification at craniotomy. This is a novel application of endovascular technology with broad potential uses in preoperative brain mapping. For example, as a resection proceeds, the brain tends to shift into the resection cavity and resembles the preoperative scan less and less as the resection proceeds. Thus, it is least accurate at the end of the case, when final decisions as to the limit of resection are most crucial. The brain mapping technique of the invention eliminates such concerns, beginning with minimal invasive clinical testing of the patient. Testing is followed by embolization at the same setting, if necessary, and clear marking of safe resection parameters that will be clearly visible to the neurosurgeon at the time of surgery.

The present invention provides endovascular brain mapping that will define safe margins of resection at craniotomy, as well as any expected neurologic deficit. Selective disruption of the blood brain barrier followed by selective infusion of a dye such as fluorescein or FD & C Green #3 can produce selective staining of the desired vascular distribution as would be expected.
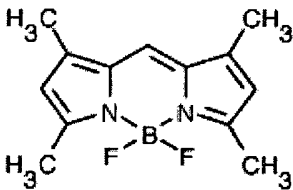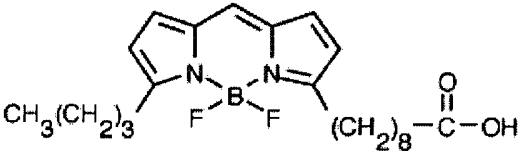
Following are examples of additional strategies for effecting preoperative endovascular brain mapping.
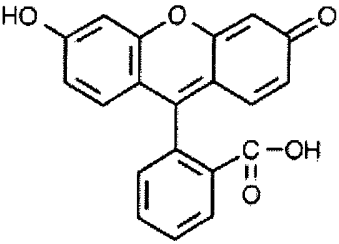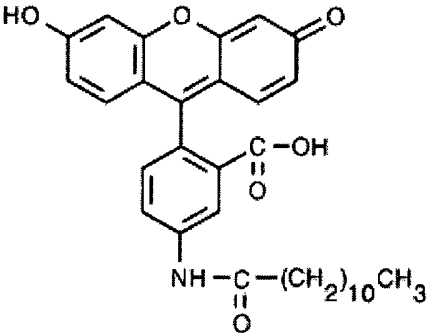
EXAMPLE 3

Passive Transport of dye (non-invasive strategy)

Dye molecules (mapping agents) which are sufficiently small (molecular weight less

than about 500) can cross the blood-brain-barrier (BBB) and reach brain parenchyma by

simple diffusion process. Small lipophilic dye molecules such as fluorescein and 4,4-

difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), their alkyl derivatives and other similar

dye molecules can be used for this purpose. Some representative examples are given below in

Table 1.

Table 1: Fluorescent compounds (fluorophores) to be investigated as mapping agents.

Fluorophores ($\lambda_{exc}/\lambda_{em}$)	Chemical Structure	Solubility

| 4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene [Tetramethyl-BODIPY® (505/515)] | | Cyclodextrins (CD) |
| 5-butyl-4,4-difluoro-4-bora-3a,4a-diaza- s-indacene-3-nonanoic acid [C₄-BODIPY® (500/510) C₉] | | CDs |

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Fluorescein		CDs
5-dodecanoylamino-fluorescein		CDs

Because of their low solubility in water, some dyes are solubilized in cyclodextrin

(CD) in PBS solution. Omnipaque™, a radio-opaque contrast agent is mixed thoroughly with

the formulation before injection in order to visualize under a fluoroscope. Initially, the

formation of an inclusion complex of lypophilic dye derivative and the CD is studied

spectroscopically to determine optimal composition. Cyclodextrins (Figure 3c, Table 2) are

sugar molecules having the structure of a hollow truncated cone with a hydrophobic cavity.

They (host) are capable of encapsulating appropriately sized fluorophores (guest) in their

hydrophobic cavity by forming inclusion complexes. Upon inclusion of a fluorophore, CDs

offer a more protective microenvironment and generally enhance the fluorescence of the

guest molecule by shielding the excited species from quenching and non-radiative decay

processes that occur in bulk solution. In general, the guest molecule loses its solvation sphere

upon entering the cyclodextrin cavity, and solvent molecules are simultaneously expelled out

from the cavity. Based on the above complex formation mechanism, dye derivatives were

selected. The α-CD and unmodified β-CD were excluded because of their small cavity size

and low water solubility, respectively (Table 2). 2-hydoxypropyl-β-CD (HP-β-CD, solubility

in water: 45 % (w/v)), γ-CD (Figure 3c), 2-hydoxypropyl-γ-CD (HP-γ-CD, solubility in

water: 450 mg/mL), carboxymethyl-β-cyclodextrin (CM-β-CD, solubility in water:

50 mg/mL) and carboxyethyl-γ-CD (CE-γ-CD) are suitable candidates for derivative/CD

complex formation and they may be lyophilized to produce freely soluble powders. A higher

degree of complexation with chemically modified CDs is to be expected due to their high

water-solubility. CD solutions at concentrations of 5, 10, 15 % (w/v) in saline and differing

amounts of dye to be incorporated (working below the saturation level) may be studied to

optimize the concentration of CD and/or dye for in vivo brain staining. The dye content of the

aqueous phase can be determined by using HPLC. The inclusion complexes can be fully

characterized by nuclear-magnetic resonance (H-NMR), UV-VIS and fluorescence

spectroscopy and their composition may be optimized to obtain maximum fluorescence

intensity.

Table 2: Characteristics of α-, β- and γ-CDs.

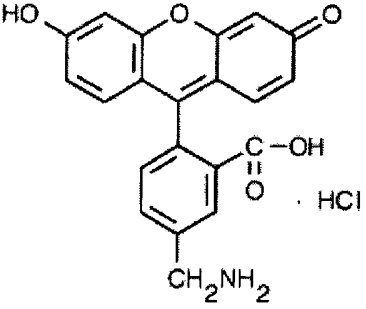|	α-CD	β-CD	γ-CD
Number of glucose units	6	7	8
Molecular weight	972	1135	1297
Solubility in water, g 100 mL^{-1} at room temperature	14.5	1.85	23.2
Cavity diameter, Å	4.7-5.3	6.0-6.5	7.5-8.3
Height of torus, Å	7.9±0.1	7.9±0.1	7.9±0.1
Approx volume of cavity, Å^3	174	263	427

EXAMPLE 4

"Pro-drug" approach

Such non-invasive strategies may, for example, entail the bioreversible covalent modification of the dye to improve its physicochemical properties that result in an enhancement of BBB transport.

Some examples are given below.

Active Transport: BODIPY, Fluorescein (as shown above) and other similar fluorescent dye molecules conjugated with nutrients (amino acids, glucose, small peptide vectors etc.) that can be actively transported into the brain. Table 3 shows two representative examples where fluorescein dye is conjugated with peptide vectors.

Table 3: Fluorescein [FLUO]-peptide conjugates.

Fluorophores	Chemical Structure	Solubility
5-(aminomethyl) fluorescein, hydrochloride (say FLUO) (to be conjugated with the peptide vector)		DMF
[FLUO-L-SynB1]	FLUO-RGGRLSYSRRRFSTSTGR, 2099 Da	Phosphate buffer saline (PBS)
[FLUO-L-SynB3]	FLUO-RRLSYSRRRF, 1395 Da	PBS

The pharmacological treatment of brain diseases is often complicated by the inability of potent drugs to pass across the BBB, which is formed by the tight endothelial cell junctions of capillaries within the brain. To overcome the limited access of drugs to the brain different methods have been developed that achieve BBB penetration. Recently, it has been shown that small peptide vectors can be used to enhance brain uptake of therapeutic drugs. These peptide vectors cross cellular membranes efficiently and have been used to enhance penetration of a number of drugs into live cells. The SynB vectors are derived from natural peptides called

protegrins. They possess an amphipathic structure in which the positively charged and hydrophobic residues are separated in the sequence. They are able to efficiently cross cell membranes without any cytolytic effect. They have also demonstrated that the mechanism of transport of coupled doxorubicin involves a saturable system, which may operate via an adsorptive-mediated endocytosis. According to the present invention, dyes can be delivered to the brain parenchyma utilizing a similar strategy. The method can be characterized as a "vectorization" of the dyes utilizing the peptide vectors, e.g., vectorization with L-SynB1 (18 amino acids), L-SynB3 (10 amino acids) would significantly increase the brain uptake of fluorescein dye (Table 3).

The peptides may be assembled by conventional solid phase chemistry using a 9-fluorenylmethoxycarbonyl/tertiobutyl protection scheme and purified on preparative C18 reverse phase HPLC after trifluoroacetic acid (TFA) cleavage/deprotection. Purity of the lyophilized products can be assessed by C18 reverse phase analytic HPLC and their molecular weight checked by matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry.

FLUO-SynB Synthesis: 5-(aminomethyl)fluorescein hydrochloride (Table 3) is suspended in dimethylformamide (DMF) containing diisopropylethylamine. Succinic anhydride (1 M equivalent) dissolved in DMF is added and incubated for 20 min. The resulting fluorescein hemisuccinate is then be activated by addition of benzotriazol-1-yl-oxopyrrolidinephosphonium hexafluorophosphate (1.1 M equivalent) dissolved in DMF. The peptide is then added to the reaction mixture after 5 min of activation and left for another 20 min for coupling. Purity of the lyophilized products is assessed by C18 reverse phase analytic HPLC and their molecular weight checked by matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry.

EXAMPLE 5

Receptor-mediated Transport (receptor-mediated transcytosis)

BODIPY, Fluorescein (as shown above) and other dye molecules conjugated with

transferrin- or insulin-receptor antibodies, immunoliposomes, and the like can be transported

into the brain by this transport mechanism.

EXAMPLE 6

Adsorptive-mediated Uptake

BODIPY, Fluorescein (as shown above) and other dye molecules conjugated with

penetratin, pegellin and the like can undergo adsorptive-mediated uptake process.

EXAMPLE 7

Emulsification of agent

Beta- Catatene emulsification, organic dye emulsification, etc.

EXAMPLE 8

Embolization of brain capillaries

Described above: (NBCA + Ethiodol + Sudan Black, or Embolization with 5 micron

tantalum particles or other dyed particles

EXAMPLE 9

Grafted-nanoparticle systems as brain-mapping agents

A variety of dye-loaded nanoparticles/microspheres grafted with the above mentioned

"pro-drug" moiety can be used as mapping agents. Fluorescein, BODIPY and similar typs of

dyes loaded in biodegradable polymers e.g. polylactic acid (PLA), polylactide-polyglycolide

(PLA-PGA) copolymer nanoparticles, n-butylcyanoacrylate (NBCA) nanocapsule-particles

can be used as nanoparticulate systems.

Endothelial cells uptake particles grafted with "prodrug" moieties by endocytosis

(cellular uptake) process. They can also be strongly adsorbed onto the brain capillaries

depending on the surface functionality. The advantages of using particulate systems are:

i)   highly photostable (dye molecules are protected from the environment by polymer

matrix,

ii)   Very bright (each particle may contain about tens of thousands of dye molecules).

Some of them could be seen by naked eyes.

iii)   More versatile choice of dye molecules (non-toxic and biodegradable organic,

inorganic, optical, luminescent and other suitable types including quantum dots (CdS, CdSe,

phosphores etc. can be incorporated inside the biodegradable polymer matrix).

iv)   Stable (dyes are incorporated inside the polymer matrix. Therefore, they are stable

towards cellular enzymatic decomposition).

It will be understood by those skilled in the art that the method of the invention is

equally applicable to any type of surgical procedure involving the brain.

CLAIMS

1.   A method for diagnostically imaging a preselected region in a warm-blooded

animal having a brain situated inside a blood-brain barrier having an ambient permeability

level, the method comprising the steps:

endovascularly administering a first chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier, where the first

chemical agent is capable of increasing the permeability of the blood-brain barrier

from the ambient level to an elevated permeability level;

endovascularly administering a second chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier following the

administration of the first chemical agent, the second chemical agent being capable of

staining the preselected region of the brain to a color visibly contrasting with non-

stained portions of the brain and of passing through the animal's blood-brain barrier to

the preselected region of the brain when the blood-brain barrier is at the elevated

permeability level but not at the ambient permeability level.

2.   The method of claim 1 wherein the second chemical agent is non-toxic to the

mammal.

3.   The method of claim 2 wherein the second chemical agent is a dye.

4.   The method of claim 3 wherein the dye is fluorescein, FD & C green #3,

[List others_____]

5.   The method of claim 1 wherein the second chemical agent also comprises a

component that is radiopaque.

6.   The method of claim 5 wherein the radiopaque component is [List several

_____]

7.   The method of claim 1 wherein the first chemical agent is arabinose,

[List others_____].

8.   The method of claim 1 wherein the mammal is a human.

9.   In a method involving surgery on the brain of a warm-blooded mammal, the

improvement comprising diagnostically imaging a preselected region in a warm-blooded

animal having a brain situated inside a blood-brain barrier having an ambient permeability

level, the imaging comprising the steps:

endovascularly administering a first chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier, where the first

chemical agent is capable of increasing the permeability of the blood-brain barrier

from the ambient level to an elevated permeability level;

endovascularly administering a second chemical agent to the animal at a location

proximal to the preselected region outside the blood-brain barrier following the

administration of the first chemical agent, the second chemical agent being capable of

staining the preselected region of the brain to a color visibly contrasting with non-

stained portions of the brain and of passing through the animal's blood-brain barrier to

the preselected region of the brain when the blood-brain barrier is at the elevated

permeability level but not at the ambient permeability level.

10.   The method of claim 9 wherein the second chemical agent is non-toxic to the

mammal.

11.   The method of claim 10 wherein the second chemical agent is a dye.

12.   The method of claim 11 wherein the dye is fluorescein, FD & C green #3,

[List others_____]

13.   The method of claim 9 wherein the second chemical agent also comprises a

component that is radiopaque.

14.   The method of claim 13 wherein the radiopaque component is [List several

_____]

15.   The method of claim 9 wherein the first chemical agent is arabinose,

[List others_____].

16.   The method of claim 9 wherein the mammal is a human.

17.   The method of claim 1 or 9 including the step of testing the preselected region

of brain for neurological deficit.

18.   The method of claim 17 wherein the preselected region of brain is tested for

neurological deficit by anesthetization.

19.   The method of claim 1 or 9 including the step of resecting the preselected

region of brain.

20.   The method of claim 1 or 9, including endovascularly isolating the preselected

region of brain from the remainder of the brain.

21.   The method of claim 20 wherein the preselected region of brain is

endovascularly isolated by embolization.

22.   The method of claim 21 wherein embolization is enabled by administration of

an embolization agent with the second chemical agent.

23.   The method of claim 22 wherein the embolization agent is Butyl
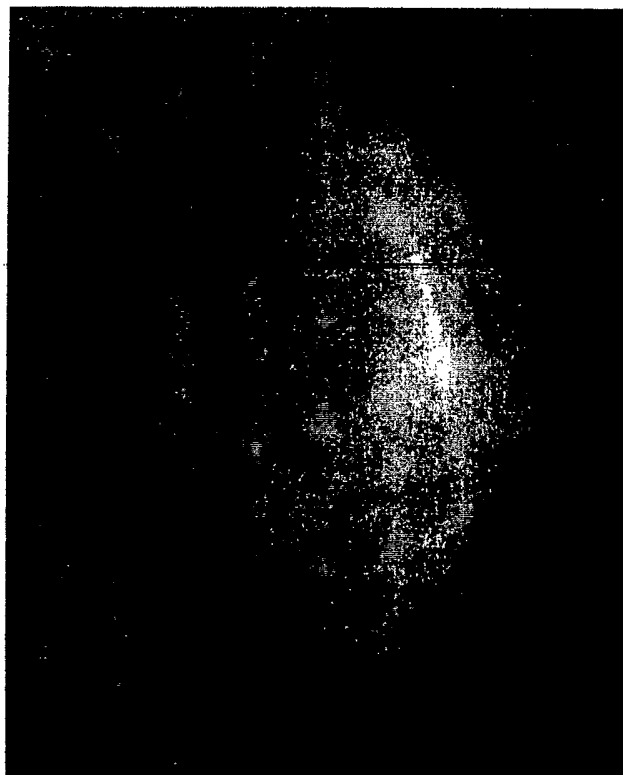
cyanoacrylate, tantalum [list others_____

_____].

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24.   A pharmaceutical composition for endovascular brain mapping in a kit form

comprising:

a first chemical agent capable of increasing the permeability of the blood-brain barrier

from an ambient level to an elevated permeability level, and a second chemical agent

capable of staining a preselected region of the brain to a color visibly contrasting with

non-stained portions of the brain and of passing through the animal's blood-brain

barrier to the preselected region of the brain when the blood-brain barrier is at the

elevated permeability level but not at the ambient permeability level.

25.   The composition of claim 24 wherein the first chemical agent is arabinose,

[List others_____].

26.   The composition of claim 24 wherein the second chemical agent is a dye.

27.   The composition of claim 26 wherein the dye is fluorescein, FD & C green #3,

[List others_____]

28.   The composition of claim 24 wherein the second chemical agent also

comprises a component that is radiopaque.

29.   The composition of claim 28 wherein the radiopaque component is [List

several

_____].

30.   The composition of claim 24 additionally containing an anesthetic.

31.   Additional embodiments of the invention relate to the following strategies for

endovascularly mapping the brain: 1) Passive transport: (a) pro-drug, (b) Modification of

mapping agent to mimic molecules that readily cross the BBB (e.g., amino acid, glucose,

etc.); 2) Active transport; 3) Receptor-mediated transport (e.g., antibody mediated); 4) Blood

brain barrier (BBB) manipulation; 5) Emulsification of agent (such as β-carotene, organic

dye, etc.) to stain capillary endothelium; 6) Embolization of brain capillaries; 7) Grafted-nanoparticle systems for brain delivery of mapping agent.
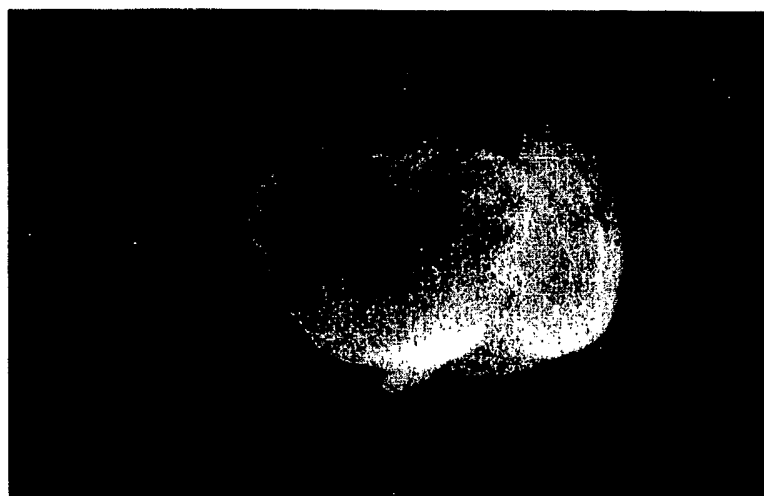
32.   Still further embodiments of the invention relate to kits for endovascularly mapping the brain and their use wherein the kits comprise, as a first component, a chemical agent being capable of staining a preselected region of the brain to a color visibly contrasting with non-stained portions of the brain and, as further components, agents for enhancing transport to the preselected regions of the brain of the chemical agent.

33.   Other embodiments of the invention relate to articles of manufacture and their use wherein the comprises packaging material and a pharmaceutical agent contained within the packaging material, wherein the pharmaceutical agent is effective for the endovascular mapping of the brain, and wherein the packaging material comprises a label which indicates that the pharmaceutical agent can be used for the endovascular mapping of the brain.

Figure 1: Fluorescein mapped rat brain; (a) dorsal view and (b) coronal section
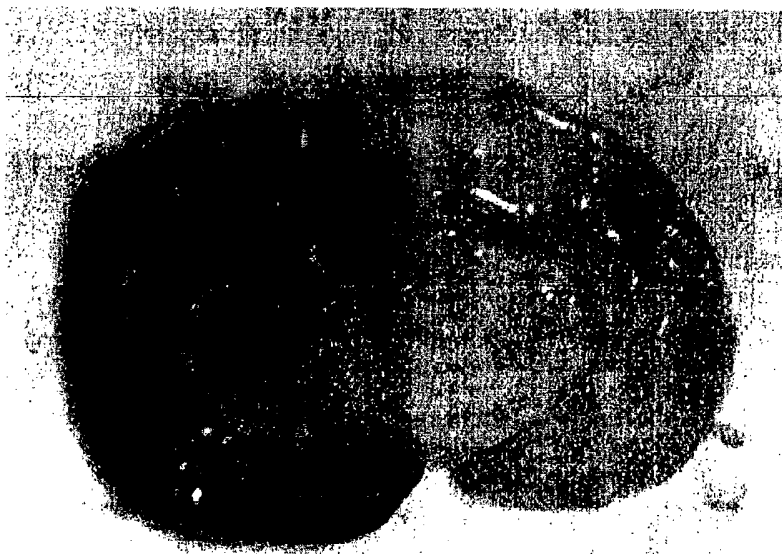

(a)

(b)

Figure 2: FD & C green #3 mapped rat brain; (a) dorsal view, (b) ventral view and (c) coronal section

(a)

(b)

(c)

Figure 3: Chemical structures of Iohexol (Omnipaque™) (a), Methohexital Sodium; 2,4,6 (1*H*, 3*H*, 5*H*) Pyrimidinetrione, 1-methyl-5-(1-methyl-2-pentynyl)-5-(2-propenyl)-, (±)-, monosodium salt (Brevital®) (b), γ-cyclodextrin (c) and doxorubicin (d).

(a)

(b)

(c)

(d)