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(71) Applicant: BRIGHAM & WOMEN'S HOSPITAL [US/US]; 75 Francis Street, Boston, MA 02115 (US).

(72) Inventors: JOLESZ, Ferenc, A.; 20 Rawson Road, Brookline, MA 02146 (US). HYNYNEN, Kullervo; 36 Oriole Street, Medfield, MA 02052 (US).

(74) Agent: POWSNER, David, J.; Choate, Hall & Stewart, Exchange Place, 53 State Street, Boston, MA 02109 (US).

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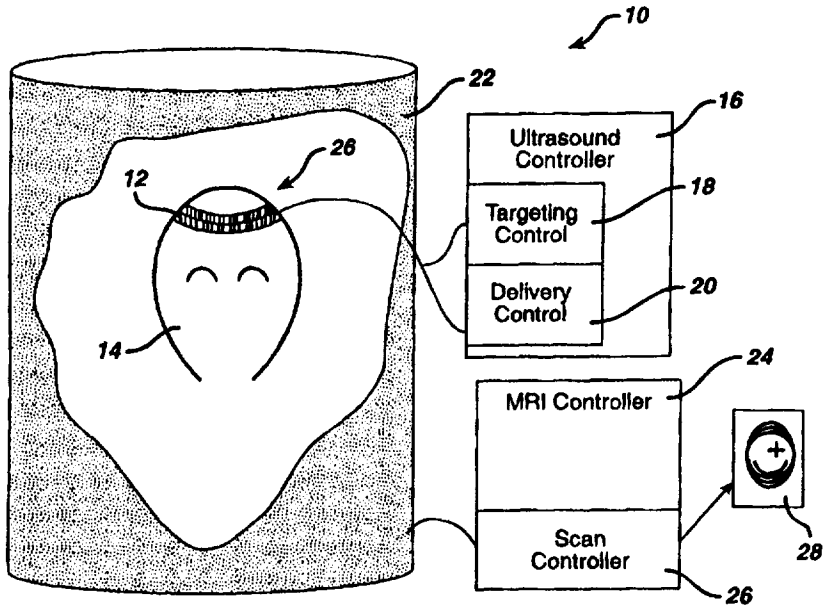

(57) Abstract

Image guide methods and apparatus for ultrasound delivery of compounds through the blood brain barrier to selected locations in the brain, target a selected location in the brain of a patient (14), and apply ultrasound to effect in the tissues and/or fluids, at that location, a change detectable by imaging. At least a portion of the brain in the vicinity of the selected location is imaged, e.g., via magnetic resonance imaging to confirm the location of that change. A compound, e.g., a neuro-pharmaceutical in the patient's bloodstream, is delivered to the confirmed location by applying ultrasound to effect opening of the blood brain barrier at that location, and thereby to induce uptake of the compound there.

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METHODS AND APPARATUS FOR IMAGE GUIDED ULTRASOUND DELIVERY OF COMPOUNDS THROUGH THE BLOOD BRAIN BARRIER

Background of the Invention

The invention pertains to medical systems and, more particularly, to methods and apparatus for delivery of compounds through the blood-brain barrier to the brain.

The blood-brain barrier prevents many compounds in the blood stream from entering the tissues and fluids of the brain. Nature provides this mechanism to insure a toxin-free environment for neurologic function. However, it also prevents delivery to the brain of compounds, such as neuropharmaceuticals, potential neuropharmaceuticals, and other neurologically active agents, that might otherwise remedy or modify neurologically-related activities and disorders.

Today, non-surgical treatments of neurologic disorders are limited to systemic introduction of compounds through the blood stream. A drawback of this form of treatment, as suggested above, is the relatively small number of known compounds that pass through the blood-brain barrier. Even those that do cross the barrier often produce adverse reactions in other parts of the body or in non-targeted regions of the brain.

Prior art surgical treatments of neurologic disorders are limited to removal or ablation of brain tissue. While these treatments have proven effective for certain localized disorders, such as tumors, they involve delicate, time-consuming procedures that may result in destruction of otherwise healthy tissues. The surgical treatments are generally not appropriate for disorders in which diseased tissue is integrated into healthy tissues, except in instances where destruction of the latter will not unduly effect neurologic function.

Patrick, *et al,* "Ultrasound and the Blood-Brain Barrier," Consensus on Hyperthermia for the 1990's (Plenum, 1990), pp. 369, *et seq.*, suggest that focused ultrasound might be used to introduce chemotherapeutic agents through the barrier.

The article is specifically directed to the use of ultrasound to modify the blood-brain barrier at targets within feline and canine brains and, thereby, to increase the barrier's permeability to a circulating dye/albumin complex. Ultrasound targeting, according to the article, is accomplished by surgically exposing the dura matter and positioning thereon a transducer/lens complex. Targets are located via stereotactic coordinates, as determined from directly-visualized or roentgenographically-visualized cranial structures. Delivery of ultrasound in the manner disclosed in the article resulted in histologically irreversible damage. Though the authors suggest that chemotherapeutic agents, such as monoclonal antibodies and immunotoxins, might also be introduced into the brain by ultrasonic modification of the blood-brain barrier, they state that further research would be necessary to determine whether permeability can be increased sufficiently for these high molecular weight compounds.

An object of this invention is to provide improved methods and apparatus for delivery of compounds to the brain, particularly, through the blood-brain barrier.

A further object of the invention is to provide such methods and apparatus as can be employed to deliver such compounds to precise locations within the brain.

A still further objects of the invention is to provide such methods and apparatus as can deliver compounds through the blood-brain barrier without surgery.

Yet another object of the invention is to provide such methods and apparatus as can deliver a full range of compounds through the blood-brain barrier.

Yet still another object of the invention is to provide cost-effective methods and apparatus for delivery of compounds through the blood-brain barrier.

Still further objects of the invention are to provide improved methods and apparatus for remedying or modifying neurological and neurologically-related activities and disorders via delivery of compounds through the blood-brain barrier.

Summary of the Invention

These and other objects are attained by the invention which provides methods and apparatus for image-guided ultrasound delivery of compounds through the blood-brain barrier to selected locations in the brain.

A method according to one aspect of the invention includes targeting a selected location in the brain and applying ultrasound to induce, in the central nervous system (CNS) tissues and/or fluids at that location, a change detectable by imaging. At least a portion of the brain in the vicinity of the selected location is imaged, e.g., via magnetic resonance imaging, to confirm the location of the change. A compound, e.g., a neuropharmaceutical, in the patient's bloodstream is delivered to the confirmed location by applying ultrasound to effect opening of the blood-brain barrier at that location (or a location based thereon) and, thereby, to induce uptake of the compound there.

In one aspect of the invention, the change induced in the CNS tissues and/or fluids by ultrasound is heating or cavitation. The location of that change is confirmed by characteristic patterns in the image. In another aspect, the change is the uptake of contrast agents (or other compounds detectable by imaging) induced at the selected location via ultrasonic "opening" of the blood-brain barrier there. Again, the location of such change can be confirmed by characteristic patterns generated during imaging. In the text that follows, the change induced in the CNS tissues and/or fluids by ultrasound is referred to as "ultrasonically-induced change," "imaging-detectable change," and similar such terms).

In a further aspect, the invention provides methods that combine the above-described targeting and delivery steps in order to introduce a compound through the

blood-brain barrier. The invention of this aspect calls for delivering a compound from the patient's bloodstream to a selected location in the brain by applying ultrasound to that location. Delivery to the selected location is confirmed by imaging the brain to confirm ultrasonically-induced change there. Particularly, where the compound is itself can be detected via imaging, confirmation is made by imaging the brain during or after delivery, and by identifying the compund's characteristic pattern at the selected location. Where the compound itself cannot be detected by imaging, confirmation is made by imaging the brain during delivery, and by identifying in the image patterns representative of ultrasonically-induced heating or ultrasonically-induced cavitation at the desired location.

In a further aspect, the invention provides methods as described above in which ultrasound is applied to the selected location in the patient's brain by surgically exposing the dura matter and by applying ultrasound through the exposed dura matter.

In a preferred aspect, the ultrasound is applied through the skull itself, e.g., via a phased array of transducers, a focused ultrasound transducer, or the combination of an ultrasound source (e.g., transducer) and an acoustic lens, placed outside the skull. According to this aspect of the invention, there is no need to perform a craniectomy or other surgical procedure on the patient.

The invention provides, in still further aspects, methods as described above in which the brain is imaged via magnetic resonance imaging, positron emission tomography, or computed tomography in order to confirm ultrasonically-induced change at the selected location in the brain.

According to further aspects of the invention, such change is ultrasonically induced in the brain at the selected location by *cavitation* and, particularly, by applying ultrasound to the selected location of the brain at frequencies ranging from 20 kHz to 5 MHz, and with sonication duration ranging from 100 nanoseconds to 1 minute.

In a related aspect of the invention, *delivery of compounds through the blood-brain barrier* is induced at the selected location by *cavitation* and, particularly, by applying ultrasound to the selected location of the brain at frequencies ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

Likewise, according to further aspects of the invention, imaging-detectable change is ultrasonically induced in the brain at the selected location by *heating* and, particularly, by applying ultrasound to the selected location of the brain at frequencies ranging from 200 kHz to 10 MHz, and with sonication duration ranging from 100 milliseconds to 30 minutes.

In a related aspect of the invention, *delivery of compounds through the blood-brain barrier* is induced at the selected location by *heating* and, particularly, by applying ultrasound to the selected location of the brain at frequencies ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

Other aspects of the invention provide methods as described above in which ultrasound is applied to the selected location in the brain at a focal region sized in accord with the volume of CNS tissue and/or fluids to which the compound is to be delivered. That region can range from 1 mm^3 - 1 cm^3.

Still further aspects of the invention provide methods as described above for image-guided ultrasonic delivery of compounds through the blood-brain barrier, where the compounds administered into the patient's bloodstream include, by way of non-limiting example, any of neuropharmacologic agents, neuroactive peptides (e.g., hormones, gastrointestinal peptides, angiotensin, sleep peptides, etc.), proteins (e.g, calcium binding proteins), enzymes (e.g., cholineacetyltransferase, glutamic acid decarboxylase, etc.), gene therapy agents, neuroprotective or growth factors, biogenic amines (e.g., dopamine, GABA), trophic factors to brain or spinal

transplants, immunoreactive proteins (e.g. antibodies to neurons, myelin, antireceptor antibodies), receptor binding proteins (e.g., opiate receptors), radioactive agents (e.g., radioactive isotopes), antibodies, and cytotoxins, among others.

Related aspects of the invention provide methods for treating neurological disorders by image-guided ultrasonic delivery of compounds through the blood-brain barrier in accord with the methods described above. Such disorders include tumors, cancer, degenerative disorders, sensory and motor abnormalities, seizure, infection, immunologic disorder, mental disorder, behavioral disorder, and localized CNS disease, among others.

In still further related aspects, the invention provides methods for modification of neurologic and neurologically-related activity (e.g., behavioral activity, memory-related activity, and sexual activity, among others) by such methods.

The invention provides, in still further aspects, an apparatus for image-guided ultrasonic delivery of compounds through the blood-brain barrier.

Such an apparatus, according to one aspect of the invention, includes an ultrasound source and a targeting mechanism for applying ultrasound generated thereby to a selected location of the brain to effect change a change there that is detectable by imaging. An imaging element generates a radiologic image of at least a portion of the brain in the vicinity of the selected location and, thereby, permits confirmation of that location. The apparatus further includes a delivery mechanism for applying ultrasound to the confirmed location (or a location based thereon) to effect opening of the blood-brain barrier at that location and, thus, to induce delivery there of a compound from the bloodstream.

By way of further example, an apparatus according to further aspects of the invention utilizes as an ultrasound source, a phased array, a focused ultrasound

transducer, or the combination of an ultrasound source and an acoustic lens, capable of applying ultrasound to the targeted location through the skull itself, without need for surgery to expose the brain.

Still other aspects of the invention provide an apparatus as described above incorporating functionality for effecting the methods described above.

These and other aspects of the invention are evident in the drawings and in the description that follows.

Brief Description of the Drawings

A more complete understanding of the invention may be attained by reference to the drawings in which:

Figure 1 depicts an apparatus according to the invention for image-guided ultrasonic delivery of compounds through the blood-brain barrier;

Figure 2 depicts an alternative configuration for an ultrasound source used in practice of the invention;

Figure 3 depicts a method according to the invention for image-guided ultrasonic delivery of compounds through the blood-brain barrier;

Figure 4 depicts an alternative method according to the invention for image-guided ultrasonic delivery of compounds through the blood-brain barrier;

Figure 5 depicts another alternative method according to the invention for image-guided ultrasonic delivery of compounds through the blood-brain barrier; and

Figure 6 depicts configurations of ultrasound sources and lenses used in practice of the invention.

Detailed Description of the Illustrated Embodiment

Figure 1 depicts an apparatus 10 according to the invention for image-guided ultrasonic delivery of compounds through the blood-brain barrier. The apparatus 10 includes an ultrasound source, shown here as a phased array of transducers 12 disposed about the head 14 of a human patient. The phased array 12 is powered and controlled by ultrasound controller 16, which includes targeting control element 18 that tunes and drives array 12 to apply ultrasound to a selected location in the patient's brain so as to effect there a change (e.g., heating, cavitation or uptake of contrast agent) that is detectable by imaging. The controller 16 also includes delivery control element 20 that tunes and drives array 12 to apply ultrasound to open the blood-brain barrier at that same location and, thereby, to induce delivery a compound from the patient's bloodstream to the brain at that location.

The apparatus 10 further includes a magnetic resonance imaging (MRI) device, comprising magnetic gradient coil and radiofrequency coil 22 and MRI controller 24, together capable of generating an image of at least a portion the patient's head (and, more particularly, of the patient's brain) to permit confirmation of ultrasonically induced change at the selected location. Controller 24 comprises scanning control functionality 26 for generating an magnetic resonance image 28 of the patient's head 14. A headholder (not shown) holds the patient's head 14 in place within the MRI tube 22, as shown.

The phased array 12 applies focused ultrasound to selected locations within the patient's brain. The array 12 can be constructed in the manner of the aperiodic ultrasound phased array disclosed in United States Provisional Patent Application No. 60/006,413, filed November 9, 1995, for APERIODIC ULTRASOUND PHASED ARRAY, assigned to the assignee hereof, the teachings of which are incorporated herein by reference.

Phased array 12 is operated in accord with the teachings herein to deliver ultrasound, through the patient's skull, in doses suitable for inducing non-destructive imaging-detectable change (e.g., heating, cavitation or uptake of contrast agent) and/or non-destructive opening of the blood-brain barrier at selected locations within the brain.

In alternate embodiments, a focused ultrasound transducer, or the combination of an ultrasound source and an acoustic lens, is substituted for the phased array 12 as a means of generating ultrasound and applying it to the brain. In such alternate embodiments, the focused ultrasound transducer, or source/lens combination, is mechanically moved in order to target differing locations within the brain (as opposed to the phased array which is aimed "electronically"). The design of such transducers and acoustic lens, is well known in the art.

More particularly, under control of targeting control 18, phased array 12 delivers ultrasound to a selected location in the brain to heat or to cause cavitation in the tissues, fluids and other structures there sufficient to induce imaging-detectable change at that location, i.e., change in the CNS tissues and/or fluids that can be detected in images generated by the illustrated imaging device. That change may constitute direct heating or cavitation of the tissues and/or fluids or, alternatively, it may constitute the uptake of contrast agent induced by opening the blood-brain barrier at the selected location. The direct inducement of imaging-detectable change is discussed immediately below. Inducement via the uptake of contrast agent is discussed later, in connection with Figure 4.

In a preferred embodiment of the invention for use with human patients, direct non-destructive heat-based imaging-detectable change is induced at the selected location in the brain applying ultrasound to the selected location of the brain at frequencies ranging from 200 kHz to 10 MHz, and with sonication duration ranging from 100 milliseconds to 30 minutes.

Likewise, direct non-destructive cavitation-based imaging-detectable change is induced at the selected location in the brain applying ultrasound to the selected location of the brain at frequencies ranging from 20 kHz to 5 MHz, and with sonication duration ranging from 100 nanoseconds to 1 minute. In contrast to imaging-detectable changes induced by heating, those induced by cavitation occur at higher peak intensity levels within this range.

Likewise, under control of delivery control 18, phased array 12 delivers ultrasound to the selected location in the brain (or a location based thereon) to heat or to cause cavitation sufficient to open the blood-brain barrier, thereby, effecting uptake of neuropharmaceuticals, potential neuropharmaceuticals or other compounds in the blood into that location of the brain.

In a preferred embodiment of the invention for use with human patients, non-destructive heat-based opening of the blood-brain barrier is induced at the selected location in the brain applying ultrasound to the selected location of the brain at frequencies ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

Likewise, non-destructive cavitation-based opening of the blood-brain barrier is induced at the selected location in the brain applying ultrasound to the selected location of the brain at frequencies ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

A further appreciation of the ultrasound dosing levels for opening the blood-brain barrier may be attained by reference to the article supplied in the Appendix I hereof and, particularly, to teachings therein with respect to the effect of differing ultrasound pulse intensities on blood-brain barrier permeability. That article, and those teachings in particular, are incorporated herein by reference.

The magnetic resonance imaging device, including MRI device 22 and MRI controller 24, comprises a conventional, commercially available MRI device. The device is operated in the conventional manner known in the art in order to generate images 28 of the patient's head (and, particularly, of the brain) in accord with the teachings herein.

It will be appreciated that any device permitting determination of the location of change in the CNS tissues and/or fluids effected by the phased array 12 (e.g., under control of targeting control 18) in the patient's brain may be substituted for the magnetic resonance imaging device. Preferably, however, the substituted device is itself a radiologic imaging device, such as, by way of non-limiting example, a computed tomography (CT) imaging device, positron emission tomography (PET) imaging device. In further embodiments of the invention, other medical imaging devices capable of detecting, distinguishing and/or locating tissues, fluids, masses, structures, substances, conditions, and other features (naturally occurring or otherwise) within the human body and, particularly, within the head and brain, are used in place of MRI, CT or PET imaging devices. These other medical imaging devices include, by way of non-limiting example, ultrasound imaging devices, X-ray imaging devices, and gamma camera imaging devices, among others.

To this end, as used herein the terms "image," "radiologic image," and the like, refer to results (whether or not human readable) generated by MRI, CT or PET imaging devices, or by such other imaging devices for use in detecting, distinguishing and/or locating tissues, fluids, masses, structures, substances, conditions, and other features (naturally occurring or otherwise) within the human body and, particularly, within the head and brain. Likewise, the terms "radiologically imaging," "imaging" and the like refer to the act of obtaining such results.

Figure 2 depicts an alternative configuration for an ultrasound source used in practice of the invention. The source comprises an ultrasound transducer 30 in

combination with a lens 30. As above, this arrangement permits focused doses of ultrasound to be applied to target's within the patient's 14 brain for inducing non-destructive imaging-detectable changes and/or non-destructive opening of the blood-brain barrier at selected locations within the brain. The illustrated source is applied directly to the dura matter, following surgical removal of corresponding portions of the scalp and skull (as illustrated by hole 34). As above, the source is powered and controlled by an ultrasound controller 16, not illustrated.

In a preferred embodiment, an ultrasound source is used to deliver ultrasound doses through the skull, obviating the need for surgery. The source is fabricated from piezoelectric material that converts an electrical signal applied on the electrode surfaces of the material to mechanical motion of the applicator. The piezoelectric material has a backing of low (for example air) or high acoustic impedance to maximize energy output through the front surface of the applicator. The electrical signal for each transducer element is provided by a signal generator and amplified by a radio frequency (RF) amplifier. The ultrasound energy can be focused by making the piezoelectric element curved or inserting a lens in front of the applicator. In these cases a minimum of one transducer is required. By using multiple transducers enhanced focusing effect may be produced.

In the case of a phased array a number of piezoelectric elements are operating together with each of them having their own RF amplifier. The electrical signals for each element are provided by a phase shifter that introduces a proper phase shift between the driving signals so that the ultrasound waves launched by each element form a common focus at a desired locations. This phase shift is modified such that the effect of skull bone and other intervening tissues between the element and the target point is compensated for so that all of the waves come to a common focus regardless of their propagation medium. The effect of the skull and other tissues are calculated based on image information (for example CT or MRI) on its properties. Thus, the phased arrays allow elimination of phase shifts introduced by skull bone that destroys an ultrasound beam focus of a focused beam at frequencies above about 1 MHz. In addition, the phased arrays can eliminate a

movement in the focal location caused by the skull during a lower frequency sonication.

A further appreciation of the construction and operation of a system according to the invention may be attained by reference to Appendix II, filed herewith.

Figure 3 depicts a method of operating the apparatus 10 of Figure 1 in order to effect image-guided ultrasonic delivery of compounds through the blood-brain barrier. In step 40, the ultrasound source is aimed to target the selected location within the patient's brain. Particularly, in sub-step 40a, the ultrasound source is aimed at the selected location. In sub-step 40b, the ultrasound source is activated to apply a dose sufficient to directly effect imaging-detectable change in the CNS tissues and/or fluids at the selected location as described above.

In sub-step 40c, at least a portion of the brain in the vicinity of the selected location is imaged, e.g., via the imaging device shown in Figure 1, to confirm the location of the imaging-detectable change. Confirmation is made, via a human or an automated image reader, via identification of patterns characteristic of ultrasonically-induced heating or cavitation at expected locations in the image. In instances where the patterns do not appear at the expected location, sub-steps 40a - 40c are repeated with revised aiming of the ultrasound source.

In instances where the ultrasound applied in step 40b effects temporary changes in CNS function (e.g., a taste sensation, a tingling sensation, an involuntary muscle motion or cessation thereof, etc.), detection of those functional changes can also be used to confirm the selected location targeted in sub-step 40a.

Once aiming of the ultrasound source has been confirmed in step 40, the compound intended for delivery through the blood-brain barrier is administered into the patient's bloodstream, e.g., via injection, ingestion, inhalation, or other such

method. In the case of injection, the compound can be administered in the vicinity of the brain, e.g., via injection into the carotid artery.

These compounds can include, by way of non-limiting example, neuropharmacologic agents, neuroactive peptides (e.g., hormones, gastrointestinal peptides, angiotensin, sleep peptides, etc.), proteins (e.g, calcium binding proteins), enzymes (e.g., cholineacetyltransferase, glutamic acid decarboxylase, etc.), gene therapy, neuroprotective or growth factors, biogenic amines (e.g., dopamine, GABA), trophic factors to brain or spinal transplants, immunoreactive proteins (e.g, antibodies to neurons, myelin, antireceptor antibodies), receptor binding proteins (e.g., opiate receptors), radioactive agents (e.g., radioactive isotopes), antibodies, and cytotoxins, among others.

In addition, compounds to be administered into the bloodstream in step 40 can include high molecular weight complexes formed by combining relatively inert substances, such as EDTA, with neuropharmaceuticals or other substances currently known to pass through the blood-brain barrier. Due to their sizes and/or molecular configurations, such complexes are prevented from crossing the barrier, except at selected locations in the brain opened via ultrasound as described herein. Use of such complexes in connection with the invention, therefore, permits localized application of compounds that might otherwise produce unwanted effects in other parts of the brain or body.

In step 42, the compound(s) are delivered from the blood stream to the selected (and confirmed) location in the patient's brain by application of an ultrasound that effects opening of the blood-brain barrier at that location and, thereby, to induces uptake of the compound there. Ultrasound doses necessary to achieve this are discussed above.

It will be appreciated that administration of the compound in step 42 need not necessarily precede application of the ultrasound in step 44. Because the ultrasonically-opened blood-brain barrier typically permits uptake of administered

compounds for at least a short period of time, the compound 42 can be introduced into the blood stream after the barrier-opening ultrasound dose is applied.

As an alternative to applying ultrasound and delivering compounds to the confirmed location, an embodiment of the invention calls for taking at least one of these actions with respect to a location based on the confirmed location. Thus, for example, neurophysiological properties or constraints may necessitate delivering the compound (and, therefore, applying the barrier-opening ultrasound) to a location different from -- but based on -- that location targeted in step 40.

Figure 4 depicts an alternative method of operating the apparatus 10 of Figure 1 to effect image-guided ultrasonic delivery of compounds through the blood-brain barrier. In step 50, the ultrasound source is aimed to target the selected location within the patient's brain. Particularly, in sub-step 50a, a contrast agent is introduced into the patient's bloodstream, e.g., via injection, ingestion, inhalation, or other such method. In sub-step 50b, the ultrasound source is aimed to dose a selected location in the brain. In sub-step 50c, the ultrasound source is activated to apply a dose sufficient to open the blood-brain barrier at the selected location and, thereby, induce uptake of the contrast agent there.

As above, it will be appreciated that administration of the compound in sub-step 50a need not necessarily precede application of the ultrasound in sub-step 50c due to the period during which the blood-brain barrier typically remains open.

In sub-step 50d, at least a portion of the brain in the vicinity of the selected location is imaged, e.g., via the imaging device shown in Figure 1, to confirm the location of the imaging-detectable change -- to wit, the uptake of a contrast agent at the selected location. Confirmation is made, via a human or an automated image reader, via identification of patterns characteristic of the contrast agent at expected locations in the image. In instances where the patterns do not appear at the expected location, sub-steps 50a - 50d with revised aiming of the ultrasound source.

As above, in instances where the compound induced for uptake in sub-step 50c effects temporary changes in CNS function (e.g., a taste sensation, a tingling sensation, an involuntary muscle motion or cessation thereof, etc.), this can also be used to confirm the selected location targeted in sub-step 40a. To this end, the compound introduced in step 50a can be selected so as to induce such temporary changes in CNS function.

Once aiming of the ultrasound source has been confirmed, step 52 of the method calls for administration into the patient's bloodstream of the compound intended for delivery. This proceeds in the manner of step 42, described above. Further, in step 54, those compound(s) are delivered from the blood stream to the selected (and confirmed) location in the patient's brain. This proceeds in the manner of step 44, described above.

As above, an alternative to applying ultrasound and delivering compounds to the confirmed location, an embodiment of the invention calls for taking at least one of these actions with respect to a location based on the confirmed location. Thus, for example, neurophysiological properties or constraints may necessitate delivering the compound (and, therefore, applying the barrier-opening ultrasound) to a location different from -- but based on -- that location targeted in step 50.

Figure 5 depicts another alternative method of operating the apparatus 10 of Figure 1 to effect image-guided ultrasonic delivery of compounds through the blood-brain barrier. In step 60, the compound intended for delivery through the blood-brain barrier is administered into the patient's bloodstream. Optionally, a contrast agent is also be administered to the bloodstream at this time. As above, these compounds can be administered via injection, ingestion, inhalation, or other such methods.

In step 62, the ultrasound source is aimed to dose a selected location in the brain. In step 64, the ultrasound source is activated to apply a dose sufficient to open the blood-brain barrier at the selected location and, thereby, induce uptake of

the compound and optional contrast agent there. As above, it will be appreciated that administration of the compound in step 60 need not necessarily precede application of the ultrasound in step 64 due to the period during which the blood-brain barrier typically remains open.

In step 66, at least a portion of the brain in the vicinity of the selected location is imaged, e.g., via the imaging device shown in Figure 1, to confirm the location of the ultrasound dosing. If no contrast agent was administered in step 60, confirmation is made by identifying patterns characteristic of ultrasonically-induced cavitation or heating in the image. In these instances, step 66 is preferably performed concurrently with step 64. If a contrast agent was administered in step 60, confirmation is made by identification of patterns characteristic of the contrast agent at expected locations in the image. In these instances, step 66 is preferably performed subsequent to step 64.

In instances where the compound induced for uptake in sub-step 64 effects temporary changes in CNS function (e.g., a taste sensation, a tingling sensation, an involuntary muscle motion or cessation thereof, etc.), this can also be used to confirm the selected location targeted in step 62.

The methods and apparatus described in the embodiments above can be employed for treating neurological disorders by image-guided ultrasonic deliver of compounds through the blood-brain barrier. Such disorders include tumors, cancer, degenerative disorders, sensory and motor abnormalities, seizure, infection, immunologic disorder, mental disorder, behavioral disorder, and localized CNS disease, among others. For example, as an alternative to conventional functional neurosurgery, the foregoing apparatus and methods can be used to introduce selective cytotoxins into selected locations of the brain to destroy all or selected cell types there. Likewise, these apparatus and methods can be employed to introduce immunologic agents at those selected locations. Still further, they can be employed in neural pathway tracing studies using retrograde or anteretrograde

axonal transport, or in neurophysiological testing using localized delivery of activation or inhibition.

In still further related aspects, the invention provides methods for modification of neurologic and neurologically-related activity (e.g., behavioral activity, memory-related activity, and sexual activity, among others) by such methods.

Described above are methods and apparatus for image-guided ultrasound delivery of compounds through the blood-brain barrier meeting the above-cited goals. It will be appreciated that the embodiments described herein are illustrative and that other embodiment, incorporating modifications, fall within the scope of the invention.

For example, a variety of ultrasound sources may be used to practice the invention. Such sources are shown, by way of non-limiting example, in Figures 6A - 6F. Thus, Figures 6A - 6B illustrate the use of a single linear and curvilinear phased array as ultrasound sources. Likewise, Figures 6C - 6D illustrate the use of a multiple linear and curvilinear phased arrays as ultrasound sources. Figure 6E illustrates the use of a large lens to focus an ultrasound beam generated by a source (not shown). Figure 6F illustrates the use of a smaller lens to focus such a beam. Finally, figure 6G illustrates the use of a partially spherical phased array as an ultrasound source. As above, the beams generated by these sources may pass through the skull or through the exposed dura matter.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application for

METHODS AND APPARATUS FOR IMAGE-GUIDED ULTRASOUND DELIVERY OF COMPOUNDS THROUGH THE BLOOD-BRAIN BARRIER

APPENDIX I

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HISTOLOGIC EFFECTS OF HIGH INTENSITY PULSED ULTRASOUND EXPOSURE WITH SUBHARMONIC EMISSION IN RABBIT BRAIN *IN VIVO*

N. I. VYKHODTSEVA,[1] K. HYNYNEN[2] and C. DAMIANOU[2]

[1]Brain Research Institute, Russian Academy of Medical Sciences, Moscow, Russia, and [2]Arizona Cancer Center and Department of Radiation Oncology, University of Arizona Health Sciences Center, Tucson, AZ, USA

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Abstract—In this study, the threshold for subharmonic emission during *in vivo* sonication of rabbit brain was investigated. In addition, the histologic effects of pulsed sonication above this threshold were studied. Two spherically curved focused ultrasound transducers with a diameter of 80 mm and a radius of curvature of 70 mm were used in the sonications. The operating frequencies of the transducers were 0.936 and 1.72 MHz. The sonication duration was varied between 0.001 and 1 s and the repetition frequency between 0.1 and 5 Hz. The threshold for subharmonic emission at the frequency of 0.936 MHz was found to be approximately 2000 W cm^{-2} and 3600 W cm^{-2} for pulse durations of 1 s and 0.001 s, respectively. The threshold was approximately 1.5-fold as high at a frequency of 1.72 MHz. However, there was considerable variation from experiment to experiment. The multiple pulse experiments at a frequency of 1.72 MHz and an intensity of 7000 W cm^{-2} showed that the histologic effects ranged from no observable damage of the tissue, to blood–brain barrier breakage, to local hemorrhagic, to local destruction of the tissue, to gross hemorrhage resulting in the death of the animal. The severity of the tissue damage increased as the pulse duration, number of pulses and their repetition frequency increased. The results indicate that the end point of the tissue damage may be controlled by selecting the sonication parameters. Such control over tissue effects can have several different applications when brain disorders are treated.

Key Words: Ultrasound, Bioeffects, Cavitation, Minimally invasive surgery.

INTRODUCTION

Image-guided focused ultrasound surgery has shown promise in noninvasive destruction of deep target volumes (Cline et al. 1994; Foster et al. 1993; ter Haar et al. 1991; Hynynen 1992, 1993; Sanghvi 1991; Vallancien 1992; Yang et al. 1991, 1992, 1993). This has increased the interest in the study of the biological effects of ultrasound at high power levels.

When ultrasound interacts with tissue, the effects can be classified as those related to the absorption of acoustic energy resulting in temperature increase and those related to the mechanics of wave propagation, primarily cavitation. The thermal effects have been extensively studied and are fairly well understood (Carstensen et al. 1974; Lele and Pierce 1972; Pond 1970; Robinson and Lele 1972). However, cavitation in living tissues has not been investigated adequately and there is a need for additional information.

Cavitation can be defined as the formation, growth and activity of a bubble or population of bubbles stimulated into motion by an acoustic field. Cavitation in fluids has been studied extensively and reviewed in many articles (*e.g.*, Apfel 1981a; Flynn 1964; Neppiras 1980). It is known that, as an ultrasound beam passes through a liquid, it can cause microbubbles to grow and oscillate within the varying pressure field. When these gas bubbles pulsate in response to the ultrasound they act on the surrounding media by unique forms of radiation pressure, forces and torque, causing shearing stresses, vibration of the cell boundaries and aggregation of particles. The collapse of a bubble can generate high local pressure and temperatures (Apfel 1981b, 1982, 1986; Flynn, 1982).

Several investigators have utilized various approaches in their studies *in vitro* on cells and organisms in suspension, and *in vivo* on plants, flies and small

Address correspondence to: Kullervo Hynynen, Ph.D., Department of Radiology, Division of MRI, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA.

rodents to determine "the threshold" for bubble-associated phenomena as they occur at ultrasonic frequencies in the lower megahertz range. They observed a number of biological effects, for instance, leakage of hemoglobin from red cells caused by shearing stresses exerted by the streaming and concentrated in a thin boundary layer near the bubble (Rooney 1972), human platelet aggregation (Barnett 1979; Miller et al. 1979) and release of ATP and other substances from platelets, erythrocytes and leukocytes (Williams and Miller 1980). Alterations in membrane permeability and rupture of vacuolar membranes resulted in exposure of cytoplasmic material to the vacuole, and deleterious effects to cells were also reported (Gershoy and Nyborg 1973; Taylor and Pond 1972). Migration of cells and other particles toward the bubble vibrating in an ultrasonic field has been predicted by Nyborg and Gershoy (1974) and their analysis seemed consistent with reports of cell aggregation in blood vessels of chick embryos (Dyson et al. 1974, 1982). So far it has not been possible to determine whether similar effects are produced within living mammalian tissue irradiated by ultrasound.

One study (ter Haar et al. 1982) has demonstrated the formation of gaseous cavities in guinea pig legs during 0.75-MHz ultrasound irradiation at 680 mW cm^{-2} *in vivo*. However, this study did not include any histologic results.

In some cases, the oscillations of bubbles may become unstable and the bubbles may collapse violently, resulting in highly localized regions of damage. Lehmann and Herrick (1953) were the first to report the transient cavitation effects on mammalian tissues *in vivo*. They observed blood vessel damage in mice caused by ultrasound exposure at a frequency of 1 MHz.

One of the sites that could most benefit from focused ultrasound surgery is the brain. There have been a number of studies investigating the mechanical effects of ultrasound in brain tissue. Warwick and Pond (1968), producing local lesions in rat brains with focused ultrasound at a frequency of 3.0 MHz, found that high intensities (2500 W cm^{-2} or more) and short exposures caused small lesions that were complicated by cavitation effects in the form of disruptive voids in the tissue, almost always associated with hemorrhage. Fry et al. (1970) described "cavitation lesions" in cat brains produced at a frequency of 1.0 MHz and at a peak intensity of 5000 W cm^{-2} at time durations of exposure between 25 and 200 ms. Lele (1977, 1987) used focused ultrasound at a frequency of 2.7 MHz and peak focal intensities of 1050–2600 W cm^{-2} to produce lesions in cat brain. He observed gross tissue fragmentation, including that of capillaries, expressed as hemorrhage, within the lesion volume in 50% of instances at a focal intensity of 1680 W cm^{-2} and in all instances at intensities of 1840 and 2600 W cm^{-2}. These sonications also resulted in a strong subharmonic and wideband noise emission that correlated with histologic observations of mechanical tissue damage.

Using a defocused ultrasound beam at an intensity of 4000 W cm^{-2}, a pulse width of 0.3 s and a pulse period of 1.0 s, Ballantine et al. (1960) found that the blood–brain barrier could be modified without damaging the surrounding parenchyma. The effects on the blood–brain barrier were shown by heavily stained parenchyma with vital dye without evidence of discrete lesions. Barnard et al. (1956) and Fry et al. (1957) reported that it was possible to produce complete destruction of the nervous tissue elements without damage to the circulatory system in that area. In small lesions (noncavitation), even though all parenchymal elements were destroyed, capillary vessels might remain intact.

Previous brain studies have shown that different types of tissue damage can occur during high power ultrasound exposure, generating cavitation in the brain. These include mechanical fragmentation of the tissue, hemorrhage as a result of blood vessel damage as well as local disturbance of the blood–brain barrier. We hypothesize that these effects on brain tissue can be separated from one another by controlling the exposure conditions, and thus, different biological effects can be selectively used for therapeutic purposes. This is of major importance for therapy, because the destruction of cells, for example, could be used during trackless ultrasound surgery of the brain. Similarly, controlled local damage and increased permeability of the blood–brain barrier without anatomical evidence of vascular damage could have significant potential in aiding chemotherapeutic agents to reach cancer cells.

MATERIALS AND METHODS

Equipment

For generation of the ultrasound fields, spherically curved piezoceramic transducers with resonant frequencies of 0.936 and 1.72 MHz, diameters of 80 mm and curvature radii of 70 mm were used. High frequency generators were constructed to drive the transducers with maximum power output of 1500 and 250 W at 0.936 and 1.72 MHz, respectively (Andreev Acoustic Institute, Moscow). Pulse duration and pulse repetition frequency could be varied from 0.0001 to 1.0 s and from 0.1 to 5.0 Hz, respectively. The acoustic output of the transducer was controlled by altering the voltage output of the generator. The transducer tested was mounted on the X–Y–Z positioner of a stereo-

taxic apparatus, and could be positioned accurately to within 0.01 cm in each of the three mutually perpendicular rectilinear planes.

Ultrasound calibration

The total acoustic power as a function of the applied electrical power was measured using a radiation force technique with a laboratory balance. The acoustic power output was measured throughout the whole range of powers used in the experiments. The relative acoustic pressure distributions at low power level were obtained by scanning a hydrophone and a focused receiver along the axis and across the focal plane of the focusing transducer (Dmitriev 1987; Gavrilov et al. 1988). The intensity at the focus was obtained by integrating the total acoustic power over the theoretical field distribution across the focus. The theoretical and experimental beam profiles gave peak intensity values accurate to within 10%. The peak spatial intensity at the focus in the rabbit's brain was calculated from the equation: $I(\text{tissue}) = I(\text{water})\, e^{-2\alpha h}$, where α is the attenuation coefficient ($= 0.024\, f^{1.18}\ \text{cm}^{-1}$) (Goss et al. 1979), f is the ultrasound frequency (MHz) and h is the focal depth (cm) in the tissue. The effects of nonlinear propagation were not taken into account in the intensity estimation. The nonlinear propagation of the ultrasound beam may reduce the actual intensity at the focus using these high power levels.

Monitoring of cavitation

Acoustic emission from the treated area was monitored with an in-house–manufactured wideband ultrasound receiver (cylindrical piezoceramic hydrophone, 1.2 mm in length, with external and internal diameters of 1.2 and 0.8 mm, respectively) (Gavrilov and Tsirulnikov 1980; Romanenko 1969). The output of the receiver was displayed on an oscilloscope after passing through a narrowband filter centered at the half-harmonic emission frequency. A bandwidth of 10 kHz was used in the time-domain studies. The oscilloscope display was recorded on film.

The half-harmonic sound emission was used as indicative of the occurrence of cavitation (Coakley 1971; Eller and Flynn 1969; Hynynen 1991; Lele 1987; Neppiras 1969).

Animal experiments

Experimental set-up. All experiments reported in this article were performed in adult rabbit brains *in vivo*. In preparation for irradiation with ultrasound, the animals were anaesthetized with nembutal (40 mg/kg body weight), shaved and positioned in the stereotaxic apparatus. The animal was lying on a temperature-controlled blanket to maintain body temperature. A detachable pointer was fixed to the transducer holder. The length of the pointer was equal to the radius of curvature of the piezoceramic spherical transducer. By means of the pointer the focal region of the acoustic beam was located with respect to the dura, which had been previously exposed by removing a 4 cm² piece of the skull. After removing the pointer, the transducer was repositioned so the center of the focal region of the beam was 11 mm below the surface of the dura. The transducer had a truncated cone with an opening closed by a polyethylene membrane. The membrane performed a double function of retaining degassed deionized water within the cone and making contact with the dura through the degassed deionized saline filled into a coupling pan attached from its lower rim to the scalp of the animal. Figure 1 shows the sonication system. Each animal was irradiated at several locations (four to six in each hemisphere) spaced by 3 mm and from 2 to 6 mm from the midline.

Experiment 1. The intensity threshold for cavitation was studied in brains of 24 adult rabbits (12 sonicated at a frequency of 0.936 MHz and 12 at frequency of 1.72 MHz). Measurements of acoustic emissions at the subharmonic frequency were made for sonications at different intensities for pulse durations between 0.001 and 1.0 s. The intensity of the ultrasound pulse for each single pulse duration was increased progressively in consecutive sonications which were separated by an interval of at least 30 s. The occurrence of the lowest amplitude subharmonic emission in 10 consecutive pulses was defined as the threshold for cavitation. This experiment was repeated in 10 different sites in at least three animals for each intensity and pulse duration investigated.

Experiment 2. To study the effects of cavitation

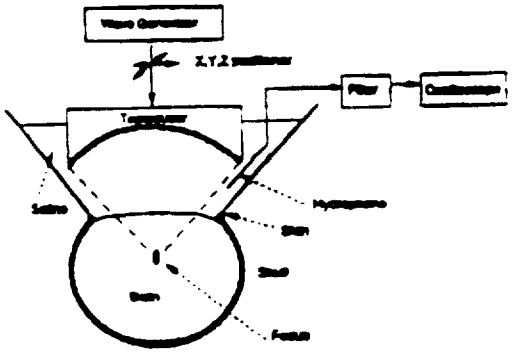

Fig. 1. Block diagram of the experimental setup: 1—ultrasound generator, 2—transducer, 3—receiver, 4—narrowband amplifier, 5—oscilloscope.

Table 1. The number of single pulse and multipulse sonications done with different pulse durations.

	Number of exposures	
Pulse duration (s)	Single pulse	Multipulses
0.01	10	28
0.05	10	28
0.1	10	81
0.5	20	5

on histologic changes in brain tissue. 63 adult rabbits were irradiated with short pulse durations of 0.01, 0.05 and 0.1 s at a frequency of 1.72 MHz and peak focal intensity in tissue of 7000 W cm^{-2}. This is higher than the cavitation threshold but lower than the lesion threshold dosage level for these single pulse durations. In each animal, two (26 rabbits) or four (37 rabbits) single and multipulse exposures were placed in thalamus opticus (nuclear gray matter) and capsula interna (white matter). A stereotaxic atlas of the rabbit's brain (Fifkova and Marsala 1969) and a rectilinear stereotaxic apparatus were used for selection and location of irradiation targets. The pulse repetition frequency was between 0.1 and 5.0 Hz and 1 to 35 pulses were given. Pulse duration exposures (suprathreshold lesion dosage level) of 0.5 s were made in brains of 12 animals (see Table 1 for number of exposures for each pulse duration).

Animal preparation after the sonications. The Bakay et al. (1956) technique was followed using trypan blue. A dose of 0.1 g/kg of body weight was prepared immediately before injection in 0.45% NaCl amounting to about 10 mL of solution, boiled and filtered. Intravenous injection of the solution was made immediately after sonication. The blue coloration resulted from ultrasonically produced breakdown of the blood–brain barrier. Thus, it facilitated the identification of the lesions in unstained frozen sections and yielded information concerning the state of the blood–brain barrier within the irradiated area.

When the animals were killed under nembutal anesthesia 4 h to 7 days after the sonication, the arterial system of the rabbit was perfused with 90 to 200 mL of isotonic saline through the aorta. After saline perfusion, the brain was also perfused with neutral 10% formalin, immediately removed and stored in fixative solution (10% buffered formalin).

All animal experiments were carried out in accordance with institutional guidelines.

Histologic preparation

Brains were frozen and serially sectioned at 0.02-mm intervals in a frontal plane. The dimensions of the lesions were determined directly in serial sections at intervals of 0.1 mm by measurement of the extent of discrete, intense trypan blue staining 24 h after euthanasia, not allowing for removal of trypan blue and shrinkage due to fixation. There was evidence of an additional zone of edema surrounding the large lesions and showing faint blue coloration. Bakay et al. (1956) found that the effect was reversible and subsided with the regression of the edema. Thus, the dimensions of the lesions were measured without the extent of the edema.

For histologic examination the brains of the 14 rabbits (12 at 24 h after euthanasia and 2 after 7 days) were stained with cresyl violet, or according to the methods of Masson for glia and collagen fibrils, Spielmeyer for myelin sheaths and Bielschowsky for axons.

RESULTS

Subharmonic emission threshold

The thresholds for the onset of subharmonic emission from the rabbit's brain sonicated *in vivo* at frequencies of 0.936 and 1.72 MHz are presented in Fig. 2. No acoustic emission at half-harmonic frequency was observed below a certain threshold intensity (which was dependent on the pulse duration as well as on the frequency of ultrasound). It occurred only sporadically at near-threshold intensity levels and appeared to be essentially dependent on the presence of cavitation nuclei or "weak spots" in the sonication area. The threshold for subharmonic emission at the frequency of 0.936 MHz was found to be approxi-

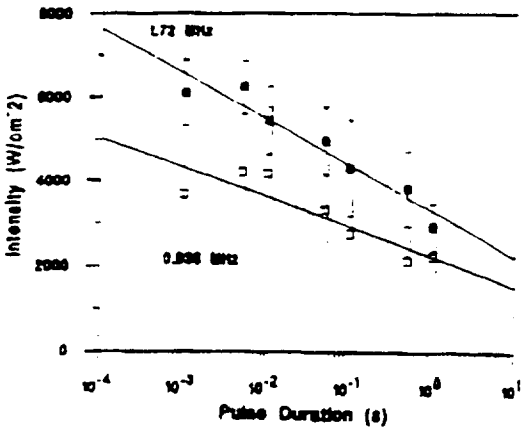

Fig. 2. The relationship between single pulse duration and peak intensity in tissue for occurrence of subharmonic emission from rabbit brains *in vivo* at the sonication frequency of 0.936 MHz and 1.72 MHz. The horizontal lines are fitted to the data using a least-squares method.

Table 2. Effect of pulse repetition frequency and number of pulses on histologic changes in irradiated area.

Number of pulses	Pulse repetition frequency (Hz)			
	0.1	0.5	1.0	5.0
2			(−), Les. 2/3	
3	(−), DB, 2/3	(−), Hem. 2/3		
4	(−), DB, 3/4	Hem. 3/3	Les. 2/2	
5			Les. 4/4	Les. 7/7
8		Hem. 3/3		
10		DB, 3/3	Hem. 1/3, Les. 2/3	Les. 5/5
12	DB, 3/3			
15	(−), Hem. 3/4		Les. 5/5	Les. 8/8
20		Les. 2/2	Les. 3/3	Les. 3/3

Minus sign (−) indicates absence of histologic changes. DB—diffuse bleeding, Hem—haemorrhages, les—local lesion. The figures are for the ratio between the number of observed effects and the total number of exposures (f = 1.72 MHz, I = 7000 W cm^{-2}, pulse duration = 0.1 s).

mately 2000 W cm^{-2} and 3600 W cm^{-2} for pulse durations of 1 s and 0.001 s, respectively. The threshold increased with decreasing pulse duration; however, the threshold values varied more as a function of location and from animal to animal. The threshold was approximately 1.5 times as high at the frequency of 1.72 MHz.

The threshold for half-harmonic emission was seen as a low amplitude spike or a burst of spikes occurring only during sonication, but randomly with respect to its beginning and end. If the sonication intensity exceeded a threshold value then strong, erratic emission, with a rise and fall in amplitude, was observed.

Fig. 3. Local haemorrhage in the thalamus of the left brain hemisphere of a rabbit 24 h after sonication: f = 1.72 MHz, peak intensity (I) = 7000 W cm^{-2}, pulse duration (PD) = 0.1 s, pulse repetition frequency (PRF) = 1.0 Hz, number of pulses (NP) = 20. Cresyl violet stain; ×27.

Histologic effects of pulsed sonication

Ultrasound pulses with intensities exceeding threshold for the onset of subharmonic emission were delivered to the rabbit brains. No histologic changes were detected at sites sonicated with single pulse durations of 0.01, 0.05 and 0.1 s. However, after repeating the sonications, different types of histologic changes were observed. In general, the severity of the damage was dependent on the pulse length, number of pulses and the repetition frequency.

Pulse duration of 0.01 s. Using multiple ultrasonic pulses 6 of 28 sites irradiated (pulse repetition frequency of 0.1, 0.5 and 1.0 Hz, 3 to 30 pulses) showed a diffuse blue staining with trypan blue or bright blue spots (3 to 5) dispersed throughout the irradiated area. Diffuse inhibition of the tissue consisted of a very light blue discoloration for the entire tissue, without selective staining of the cells. This indicated the penetration of the tissue by trypan blue, which escaped from the blood vessels as a result of damage to and increased permeability of the blood–brain barrier.

Pulse duration of 0.05 s. With pulse repetition frequency of 0.1, 0.5 and 1.0 Hz, and the number of pulses 3 to 10, tiny haemorrhagia, blue-spot dispersement as well as diffuse blue-stained areas (not more than 1.0 mm in diameter) could be observed in

Fig. 4. Pyknotic glia cell nuclei and dark shrunken nerve cells close to haemorrhage resulting from the sonication. Cresyl violet stain: ×650.

some cases. However, no trace of damage was found in either cell- or fiber-stained sections, in spite of clearly visible red spots or trypan blue staining on frozen blocks of the brain.

Pulse duration of 0.1 s. The pulse repetition frequency and number of pulses had an effect on the severity of the tissue damage. Diffuse blue coloration (DB), haemorrhagia (Hem) and local lesions (Les) in the irradiated area were induced with multiple pulses depending on pulse repetition frequency and number of pulses (see Table 2). Generally, the tissue damage became more severe with increasing number of pulses or/and repetition frequency. Diffuse blue coloration resulted from sonication with 2 to 12 pulses repeated at a low frequency (0.1 Hz). The area uniformly stained by trypan blue was as much as 1.0 to 1.2 mm in diameter. It had no definable boundaries and could be detected only on gross examination or by a small magnification of unstained frozen sections. No evidence of discrete lesions or traces of damage in either cell- or fiber-stained sections were observed. The capillaries looked normal within the irradiated area. The lamina were empty due to perfusion and the capillary walls showed no anatomical changes.

Haemorrhagia were clearly visible on gross examination of unstained frozen sections as red areas occasionally with additional light blueing in sites sonicated with 15 or more pulses repeated at a low frequency (0.1 Hz) or with 3 to 10 pulses repeated at higher frequencies (0.5 and 1.0 Hz). Such lesions were up to 1.5 mm in diameter and were round in shape, but in some cases the size and shape of the lesions varied. Figure 3 shows a 1.2-mm-long lesion in the thalamus. The lesion consists of some separated areas with large erythrocyte concentration throughout each of them and exhibits a curved shape, indicating a preferential orientation, perhaps due to large blood vessel placement. Between the areas occupied by erythrocytes there were areas in which erythrocytes were not present and the blood vessels appeared to be intact. All identifiable neurons showed varying degrees of abnormality. One predominant feature was shrinkage and intense stain-

Fig. 5. Local lesion in the thalamus of the right brain of the rabbit 24 h after sonication: f = 1.72 MHz, I = 7000 W cm^{-2}, PD = 0.1 s, PRF = 3.0 Hz, NP = 5. Cresyl violet stain: ×27.

ing of cell bodies and nuclei with no visible internal structure. Much cellular debris as well as a few normal glia cell nuclei were present close to the hemorrhage (Fig. 4).

Pulses repeated at a frequency of 1.0 and 5.0 Hz resulted in a trackless circumscribed lesion at the focus. The severity of histologic changes was found to be dependent upon the number of pulses. Tissue fragmentation, including that of capillaries expressed as haemorrhagia within the lesion, was found in about 30% of the sites irradiated with 2 to 35 pulses.

The lesion produced with five pulses at a frequency of 5.0 Hz in the thalamus was 1.2 mm in diameter and on gross examination could be detected as an elongated, uniformly stained area. Its longer axis was parallel to the axis of the ultrasound beam. On microscopic examination of cresyl violet–stained sections, the lesion (24 h after exposure) appeared as a uniform pale-stained area which was sharply demarcated from normal tissue (Figs. 5 and 6). This is typical of a zone of liquefaction necrosis. The neurons, axis cylinders and myelin sheaths as well as glia had completely disappeared from the central part of the lesion. The narrow peripheral part of the lesion contained a few nerve cells which showed morphological evidence of acute damage. Their nuclei were small, dark and pyknotic. The nucleoli were all but invisible and the nuclei were displaced to the edge of the cytoplasm. The cytoplasm was partly dissolved. Only a few microglia were present and began to metamorphose so that by 7 days after sonication the lesion showed phagocytic and vascular capillary infiltration indicative of the reparative process (Fig. 7).

Fig. 6. The central part of the lesion presented in Fig. 5 contains pyknotic glia cell nuclei, dark nuclei of nerve cells with shreds of cytoplasm and indistinguishable nucleoli and a few inflammatory cells. Cresyl violet stain; ×275.

Fig. 7. An ultrasonically induced lesion 7 days after sonication shows phagocytoc and vascular capillary infiltration indicative of reparative process. Large holes and clefts are seen: f = 1.72 MHz, I = 7000 W cm^{-2}, PD = 0.1 s, PRF = 1.0 Hz, NP = 30. Cresyl violet stain; ×27.

Lesions produced with 10 to 20 pulses (repetition frequency of 1.0 and 5.0 Hz) were elongated, 1.8 to 2.5 mm in length and up to 1.5 mm in diameter and homogeneously stained with trypan blue. Figure 8 shows a lesion situated in the thalamus produced with 15 pulses repeated at frequency of 5 Hz. There was a great disarray of the matrix including the central part being missing, perhaps occurring during the prepara-

tion of the sections. The [illegible] the matrix was completely disorganized. There were a large number of holes and clefts, and many of the small blood vessels appeared broken and were surrounded by areas with erythrocyte dispersement into the surrounding tissue (Fig. 9). Some blood vessels were unbroken but appeared to be dilated and congested by erythrocytes which probably were coagulated. No intact neurons appeared in any part of the lesion. Much indistinguishable cell debris, "ghost" cells, small dark and pyknotic nuclei with shreds of cytoplasm and invisible nucleoli as well as pyknotic glia cell nuclei could be observed in the lesion (Fig. 10). Single nerve cells close to the border, but outside the lesion, were also affected. The nuclei were pale and cytoplasms were vacuolated in some cells, whereas in others the cytoplasms were dark and the cell bodies shrunken. The glia cells in the vicinity of the lesion were less affected than the neurons.

Large lesions of about 1.7 mm in diameter, produced with 20 to 35 pulses of 0.1-s duration, showed a characteristic "island-moat" pattern. On gross examination the center of the lesion showed little or no vital staining, whereas the peripheral region surrounding the center was intensely stained with trypan blue. Microscopic examination showed the central zone to be coagulation necrosis and the surrounding zone to be liquefaction necrosis. Tissue fragmentation, including that of capillaries (expressed as local hemorrhage), was found in most of the lesions.

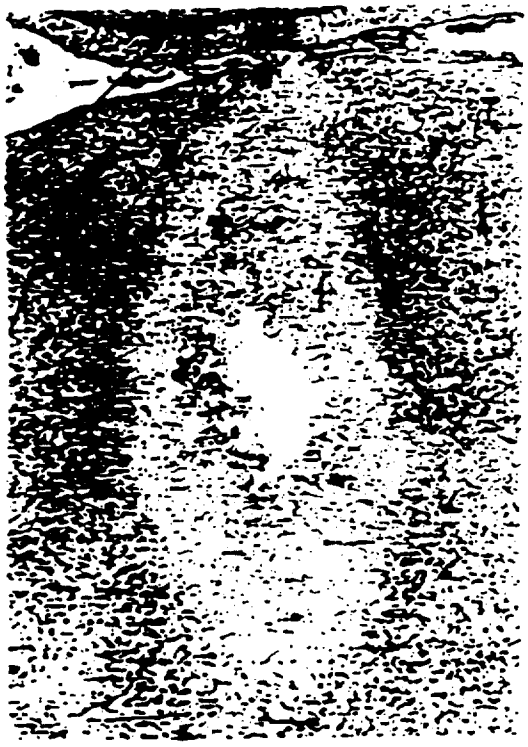

Fig. 8. A lesion in thalamus of a rabbit without the central region which appeared to be lost during preparation of sections due to gross tissue fragmentation: f = 1.72 MHz, I = 7000 W cm^{-2}, PD = 0.1 s, PRF = 5.0 Hz, PN = 15. Cresyl violet stain: ×27.

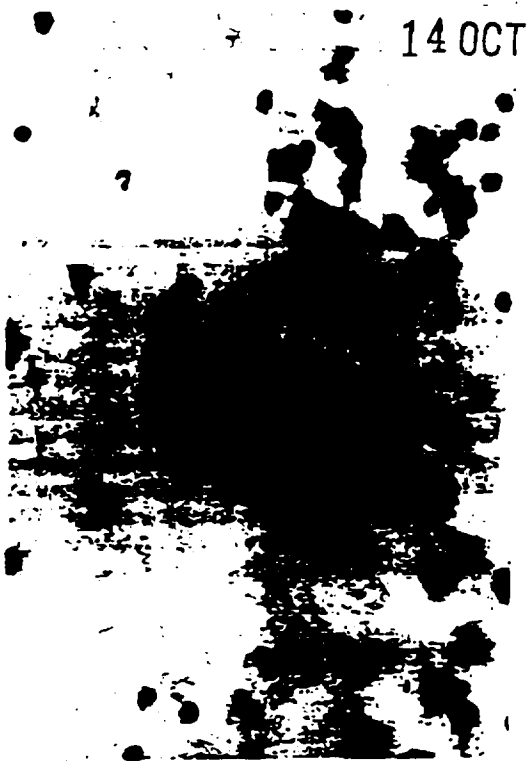

Fig. 9. Indistinguishable basophilic debris of cells and coagulated erythrocytes resulting from the sonication. Cresyl violet stain: ×440 (the same exposure as in Fig. 8).

Pulse duration of 0.5 s. Sonications with two to three pulses caused lesions with the "island-moat" pattern. Gross hemorrhage distending the lesion and spreading intracerebrally, and often rupturing into the lateral ventricles, was observed. Sonication with three pulses resulted in postoperative death of animals due to gross hemorrhage in all cases.

Occasionally "echo lesions" could be detected at the base of the brain, which could have been caused by the divergent beam absorption and reflection at the concave regions in the base of the skull.

DISCUSSION

The results presented in this article offer a new potential for cavitation-based ultrasound therapy of the brain. The results indicate that the effects of cavitation can to some extent be controlled by pulse duration, number of pulses and repetition frequency.

The four different effects observed were: modification of the blood–brain barrier; local haemorrhagia; destruction of tissue; and gross haemorrhage, resulting in death of the animal. The knowledge of these end points will be useful when target volumes in the brain are treated using focused ultrasound.

From the data presented it is obvious that strong subharmonic emission can be induced in rabbit brain *in vivo*. Based on the work of Neppiras (1969), Lele (1977) and Hynynen (1991) this is an indication of cavitation in tissue. Our histologic observations also indicated that strong mechanical damage was usually associated with subharmonic emission. The threshold of the subharmonic emission decreased slightly as the pulse duration increased. The higher frequency had a higher threshold. This trend agrees with the measurements done *in vivo* in muscle tissue (Hynynen 1991).

The actual threshold values for cavitation in brain reported here appear to be larger than those reported by others (*e.g.*, Lele 1977, 1987). The intensity threshold had significant variations from location to location, and thus it is difficult to make exact comparisons between different studies. In addition, measurement of peak intensity of sharply focused transducers is difficult. All hydrophones used are large compared to the focal diameter with the sharply focused transducers. Similarly, thermal techniques are less accurate when the focal spot is small and intensity high. The technique used here to obtain peak intensity from the measured acoustic power based on the focal distribution should have fewer uncertainties, and it typically gives higher intensity values than those observed by hydrophones when sharply focused transducers are used.

The severity of the tissue damage of an ultrasound exposure associated with strong subharmonic emission increased with increasing pulse duration, number of pulses and with the repetition frequency. The histologic observations showed that the tissue damage was mainly mechanical. The trends observed are also consistent with the cavitation mechanism. First, the increased pulse length allows the bubbles to grow and do more damage. Second, multiple pulses have an additive effect. Finally, when the repetition frequency is increased the interval between the pulses is decreased and, thus, the bubbles formed during the previous sonication have less time to dissolve. These remaining bubbles cause damage during the following ultrasound pulses.

To study the relationship between the occurrence of cavitation and damage in tissue, rabbit brains were sonicated at the spatial and temporal peak intensity of 7000 W cm^{-2} (f = 1.72 MHz). These sonications did not cause observable histologic damage despite the occurrence of subharmonic emission during single pulses of 0.01, 0.05 and 0.1-s duration. Multiple pulses of the same duration caused different types of damage depending on pulse duration, repetition frequency and number of pulses.

Only bright blue spots scattered over the irradiated area or diffuse blue-stained small areas were observed in sites sonicated with pulses repeated at low frequencies. This indicates that within the sonicated area the blood–brain barrier was altered and made permeable to trypan blue dye due perhaps to cavitation. Blue spots showed the locations in which cavitation could occur first.

We have also found tiny haemorrhage or small local haemorrhagic areas without damaging the surrounding parenchyma within sites insonated with pulses repeated with a higher pulse repetition frequency. Ballantine et al. (1960) also damaged the blood–brain barrier without

Fig. 10. Small, dark and pyknotic nuclei with shreds of cytoplasm invisible nucleoli of the severely affected neurons. Cresyl violet stain: ×1625 (the same exposure as in Fig. 8).

damaging the parenchyma as observed that erythrocytes and polymorphonuclear leukocytes appeared to penetrate parenchymal cells. Similar effects on parenchymal cells in liver were reported by Bell (1958); however, the cause of damage to the blood–brain barrier was not known. Shearing stresses and vibration of cell boundaries generated by vibrating bubbles produced by ultrasound in blood vessels, free radicals created by the transient cavitation (Al-Karmi et al. 1994; Edmonds and Sancier 1983) or rupture of cell membranes due to bubble collapse could cause the increase in membrane permeability. In any case, these experiments indicate that it may be possible to alter the blood–brain barrier reversibly or break down blood vessel walls without producing a discrete lesion by proper selection of dosage parameters.

Local lesions in the focal region of the ultrasound beam were produced with multiple 0.1-s pulses repeated at a frequency of 1.0 and 5.0 Hz. Liquefaction necrosis without fragmentation of tissue and trace of haemorrhage was detected if there were no more than five pulses. The structural changes could be assumed to be produced by combined thermal and transient cavitation effects.

Gross hemorrhage resulted in death of the animals in all cases exposed to three 0.5-s sonications. This is assumed to be caused by destruction of larger blood vessels which caused the bleeding. This is in agreement with the observation of Lehmann and Herrick (1953) who observed blood vessel damage after continuous-wave ultrasound exposure. Such bleeding should be avoided during brain therapy, and thus, the exposure conditions should be designed such that only short ultrasound pulses are utilized.

In conclusion, the results observed in this article are important because they show that different types of tissue effects can be induced by pulsed high intensity ultrasound. The effects can be separated from each other by selecting the pulse duration, repetition frequency and number of pulses. The modification of the blood–brain barrier could have significant potential for allowing chemotherapeutic agents to enter into the tumor cells. Complete damage of the cells without damage to the vasculature could be useful for surgical purposes. Finally, selective damage to the tissue vasculature could be induced by delivering, for example, 15 pulses with a duration of 0.1 s and a repetition frequency of 0.1 Hz. It is obvious that such control over the tissue effects could have significant therapeutic potential and should be studied further.

Acknowledgements—In addition to the institutional support this research was partly sponsored by a NCI Grant RO1 C46627 and a grant from General Electric Medical Systems.

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent Application for

METHODS AND APPARATUS FOR IMAGE-GUIDED ULTRASOUND DELIVERY OF COMPOUNDS THROUGH THE BLOOD-BRAIN BARRIER

APPENDIX II

Materials and Methods

Sonications:

The ultrasound was generated by using in-house manufactured focused ultrasound transducers. Three different transducers were used for this study. They were all manufactured by mounting a spherically curved 10 cm diameter piezoelectric ceramic (PZT4) bowl in a plastic holder using silicon rubber. The ceramic had silver or gold electrodes both in the front and back surface. The single element transducer had a radius of curvature of 8 cm and was connected to one coaxial. The transducer had a fundamental resonance frequency of 0.556 MHz and the third harmonic frequency of 1.67 MHZ. One coaxial cable connected the electrodes to a LC matching network (separate for each frequency) that matched the electrical impedance of the transducer and the cable to the RF amplifier output impedance of 50 ohm and zero phase. The matching circuit was connected to an ENI amplifier (both ENI A240L and A500 were used in the tests). The RF signal was generated by a signal generator (Stanford Research Systems, Model DS345).

The two phased arrays had similar structure and the same driving hardware; the resonant frequency being their only main difference. The two arrays operated at 0.6 MHz and 1.58 MHz. The radius of curvature of both of the transducers was 10 cm and both of them were cut into about 1 cm2 square elements as shown in figure 1. The total number of elements in both arrays was 64 although only 60 were driven in the experiments due to hardware limitations. The ceramic bowl was cut using a diamond wire saw so that the elements were completely separated by a 0.3-0.5 mm space. The space left by the cutting was filled by silicone rubber that kept the array elements together and isolated them acoustically from each other. The silicone rubber allowed the transducer elements to move with minimum amount of clamping. Each transducer element was connected to a coaxial cable and a matching circuit that was individually tuned. The array was driven by a in-house manufactured 64 channel driving system that included a RF amplifier and phase shifter for each channel. The phase and amplitude of the driving signal of each channel was under computer control (see Buchanan and Hynynen for detail).

Ultrasound measurements

The ultrasound pressure wave distributions were measured using needle hydrophones (spot diameter 0.5 and 1 mm) and an amplifier (Precision Acoustics Ltd). The amplified signal was measured and stored by a oscilloscope (Tektronix, model 2431L). The hydrophone was moved by stepper motors in three dimensions under computer control. The pressure amplitudes measured by the oscilloscope were stored by the computer for each location.

The absolute pressure amplitudes at the maximum power levels at the focus were measured by a shock wave hydrophone (Sonic Industries) and the oscilloscope.

Experiments:

A piece of human skull (top part of the head: front to back 18 cm and maximum width 12 cm) was obtained and fixed with formaldehyde. It has been shown that the acoustic properties of formaldehyde fixed skull and a fresh skull are almost identical. The experimental setup is shown in figure 2. The ultrasound applicator under test was positioned in a water tank the walls and bottom of which were covered by rubber mat to reduce ultrasound reflections. The tank was filled with degassed deionized water. The hydrophone that was connected to the scanning frame, was positioned to the focus of the ultrasound field. The pressure amplitude distributions were measured in the water by scanning the needle hydrophone. After the water experiments the piece of skull was positioned in front of the transducer and the ultrasound field measurements repeated. In the phased array experiments the phase shifts introduced by the skull were also mapped and corrected. This was done by positioning the hydrophone in the focal position of the ultrasound field without the skull and then driving each transducer element separately while measuring the phase of the wave with the hydrophone. From these measurements a phase correction for each transducer element was calculated and programmed in the phase shifters. Then the ultrasound field measurements were repeated while driving the array with the

corrected signals. In all of the above measurements the ultrasound field was continuously on at a low power level.

In a second set of experiments the total peak pressure amplitudes achievable in the focus through the skull were measured. In this experiment the transducer was placed on the bottom of the tank and the beam aimed up towards the water surface. The skull was placed on the transducer so that it was supported by the edges of the applicator but not by the piexoelectric elements. The shock hydrophone was lowered at the focal depth and positioned to the acoustic focus. The tranducer was used in the burst length of 10 or 20 cycles. This was done to avoid electrical interference that was picked up by the hydrophone during sonication.

Results

It was possible to produce a well focused beam with the 0.559 MHZ single element transducer through the bone. The beam had secondary peaks introduced by the skull but the main peak was the highest. The location of the peak was also shifted by the skull by 1-2 mm from its geometric position (figure 3). However, focus was completely destroyed when 1.67 MHZ was used. (Figure 3.b) Similar results were obtained with the phased array. Figure 4 demonstrates the effect of skull on the pressure amplitude distribution across the focus. The effect of the skull in the focal shape can be reduced by correcting the phase. The main impact of the phase is in the location of the focus that can be corrected back to the geometric focus. The magnitude is reduced to 26 % and 31 % of its water value without and with the phase correction, respectively. The importance of the phase correction is demonstrated more clearly with the higher frequency array. With this array the focus is completely destroyed by the skull. However, when the phase correction is introduced the focal spot is returned into its original shape.

To demonstrate the power transmission capability of the skull the peak pressure amplitude in the focus with the 0.559 MHZ single transducer was measured. The maximum pressure amplitude was 8.0+/- 0.6 MPa. The variation resulted from reposioning the peace of skull on the transducer. Similar measurements with the 0.6 MHZ phased array revealed that the RF-amplifiers could not deliver adequate power and pressure amplitudes of 1.5 MPa were measured. Higher powers could have been delivered with a higher power amplifier system.

Discussion

The results demonstrated that an ultrasound beam can be focused through the skull at frequencies around 0.6 MHz or lower with a few millimeter shift in the focal position away from its geometric focus. The secondary pressure peaks are also enhanced by the skull. These effects on the focal shape can be reduced by using phased array and correcting for the phase shifts caused by the bone. At higher frequencies the wavelengths are shorter and the propagation delay variations caused by the variable thickness of the skull become significant when compared with the wavelength. This results in destruction of the focal spot when the focused beam propagates through the skull. The results demonstrated that the effects of the skull to the beam shape can be eliminated using a phased array with proper phase corrections and sharp focusing can be achieved. In this study the phase correction was calculated from hydrophone measurements. We predict that the same corrections could be made by obtained the skull thickness from a CT scan and then calculating the phase correction required for each array element.

Our driving hardware did not permit us to deliver adequate energy through the skull to reach cavitation threshold with the phased array. However, the single element transducer at the 0.554 MHz allowed us to deliver up to 8 MPa pressure amplitudes through the skull. With a phased array the phase correction could increase the pressure amplitude to about 9.5 MPa at the same driving conditions. This value was reached through an area of 10 cm in diameter. If the whole available skull surface

around the brain is utilized then at least three times larger window could be used. Thus it is estimated that pressure amplitudes around 30 MPa can be induced in the brain through the skull. These values are significantly above the 4 MPa that was measured to be the threshold value in vivo muscle at 0.6 MHz (Hynynen 1991) and the value of 8.5 MPa at 0.936 MHz measured in vivo rabbit brain (Vykhtodseva et al., 1995). The cavitation threshold is frequency dependent decreasing with the frequency. Thus the results demonstrate that adequate ultrasound transmission through skull can be generated to induce cavitation. The pressure values at the skull or skin are well below the thermal and cavitation damage thresholds.

Although good results were achieved with only 60 transducer elements in the phased array it is likely that more and smaller elements are needed in order to be able to move the focal spot inside of the brain. Much more work needs to be done before the array geometry is optimized.

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Figures :

Figure 1. A diagram of one of the phased arrays.

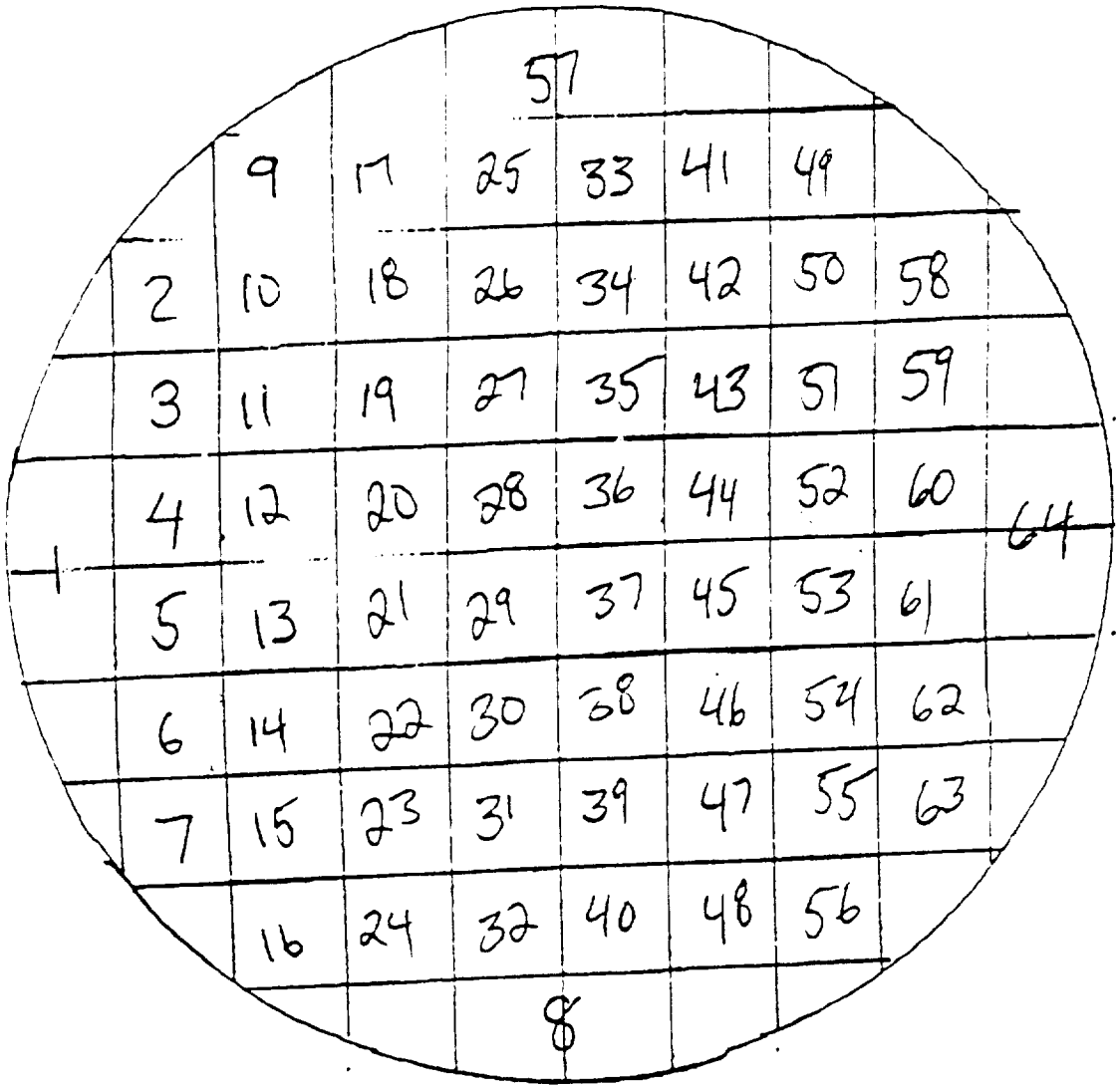
Figure 2. A diagram of the experimental set up.

Figure 3. A. The ultrasound intensity distribution across the focus of the 0.559 MHZ single transducer measured in water. B. The same distribution when the skull was in front of the transducer, C. The intensity distribution at 1.67MHz measured in water and D. through the skull.

Figure 4. A. Pressure amplitude profiles across the focus of the 0.6 MHz phased array in water, through the bone and through the bone when phase correction was used.

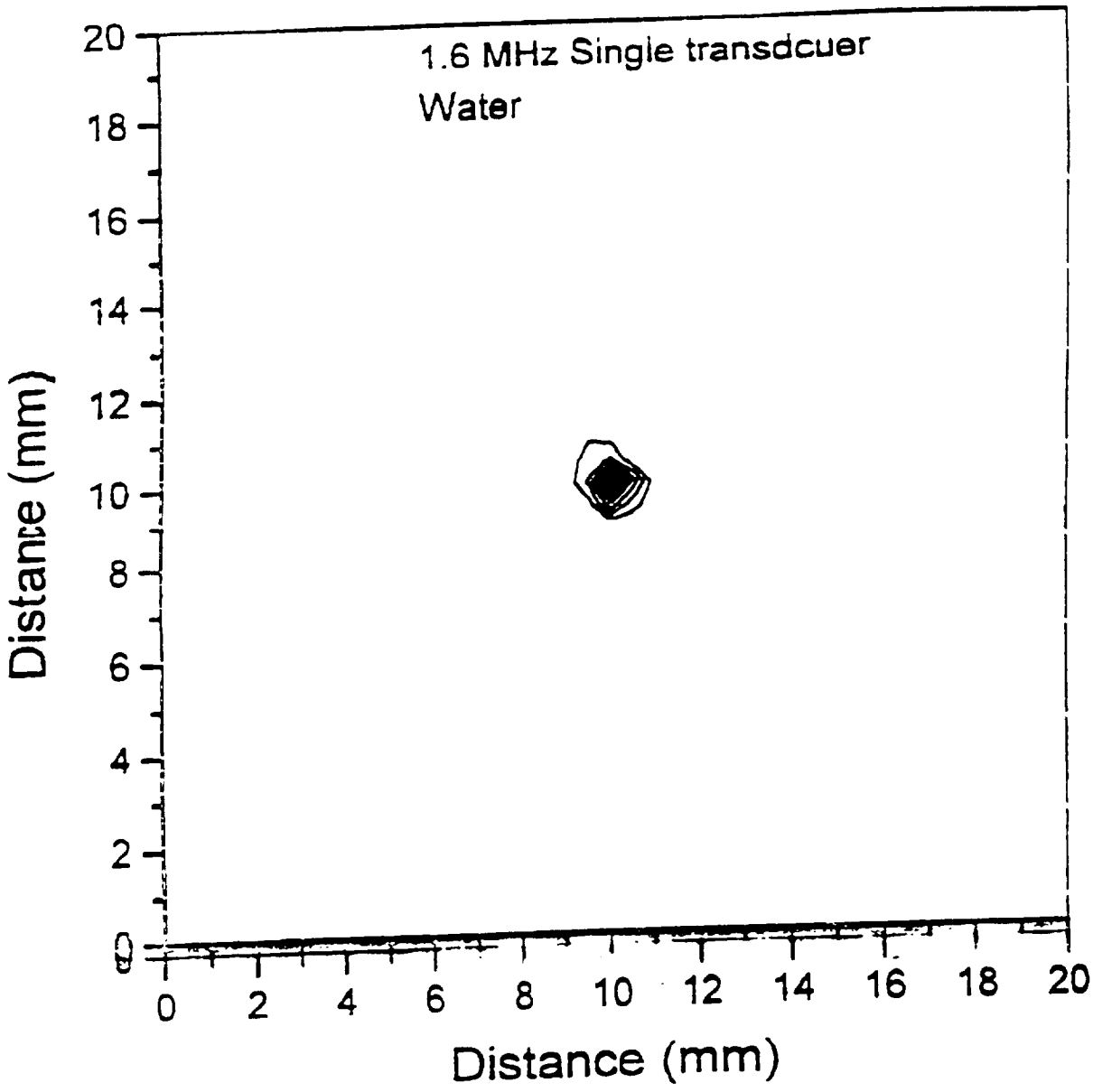
Figure 4. B. The same but along the axis.

Figure 5. The ultrasound intensity distribution measured across the focus of the 1.58 MHz phased array. A. In water.B. through skull without phase correction and C. Through skull with phase correction.

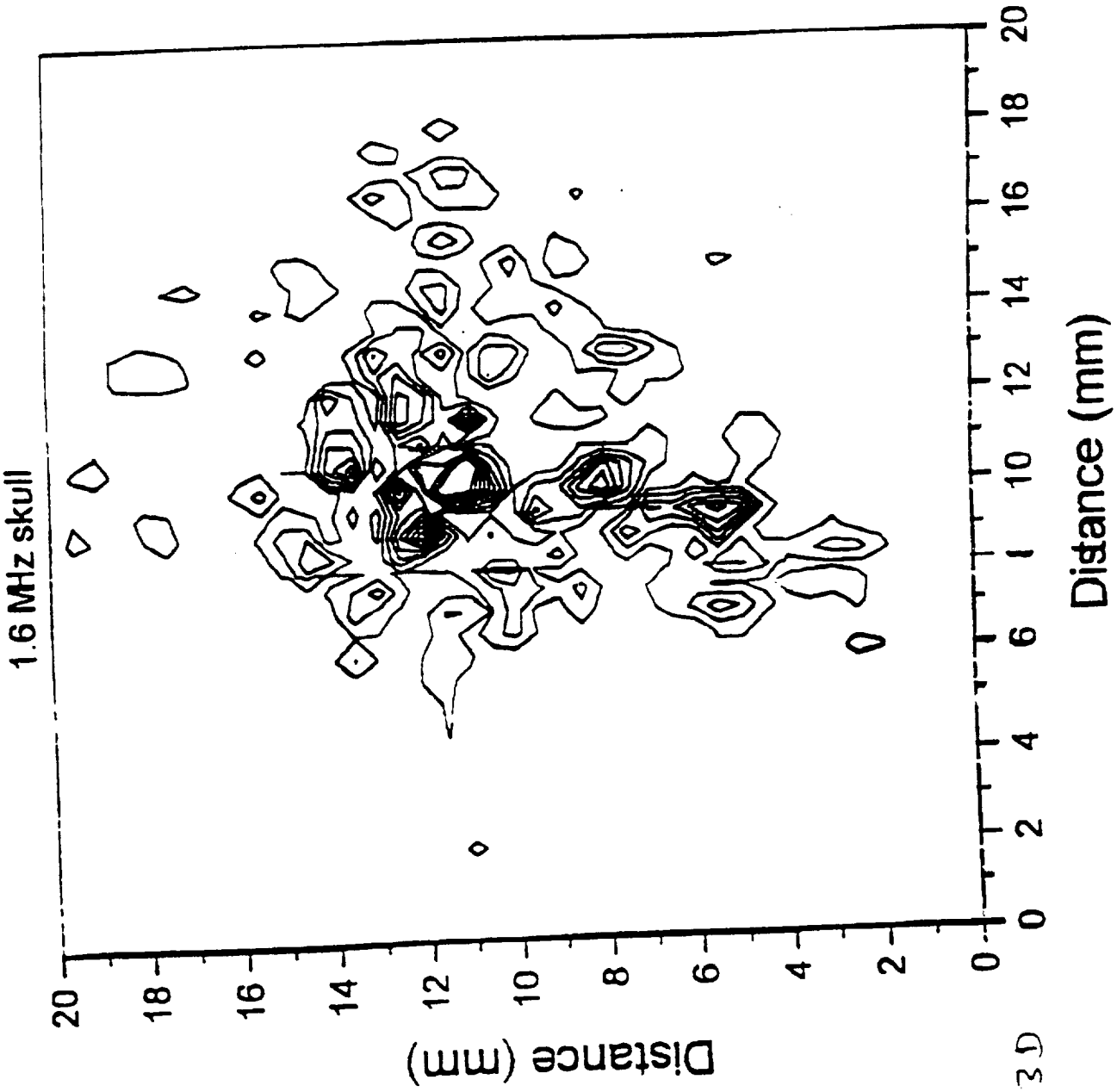


Back of Txr

Fig 1.


1.6 MHz Single transdcuer
Water
Distance (mm)
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20

3c.


1.6 MHz skull
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20

3D

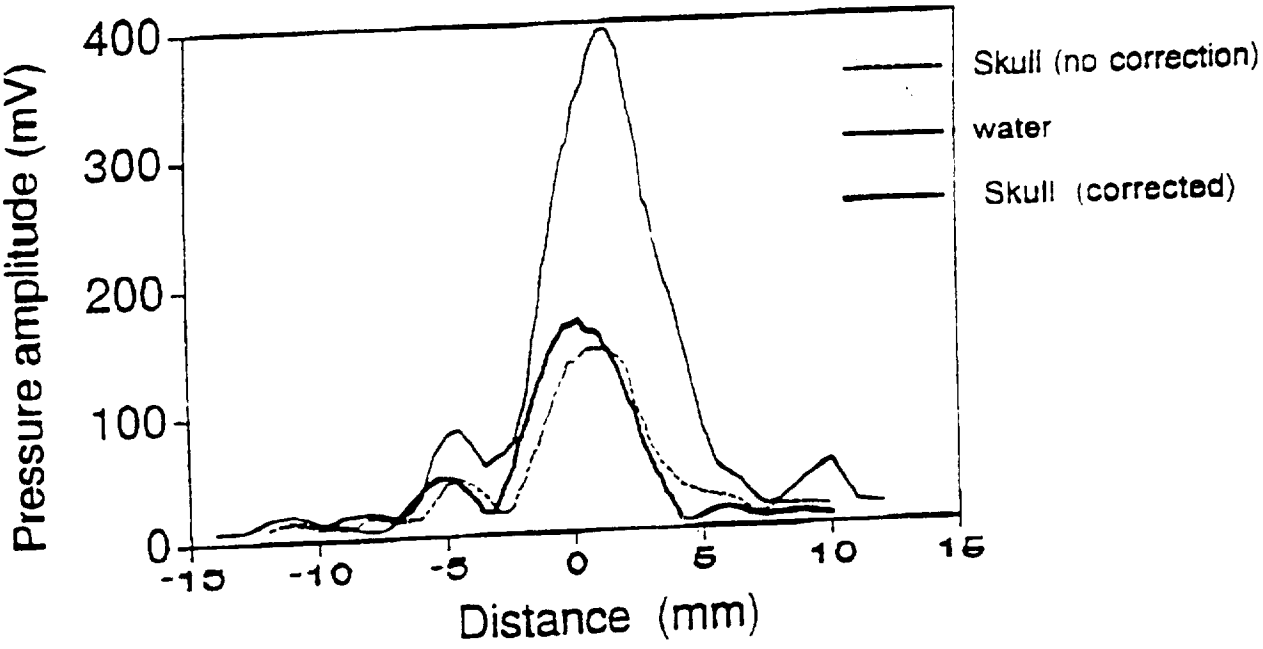

Pressure amplitude (mV)
400
300
200
100
0
-15
-10
-5
0
5
10
15
Distance (mm)
Skull (no correction)
water
Skull (corrected)

Figure 4, A

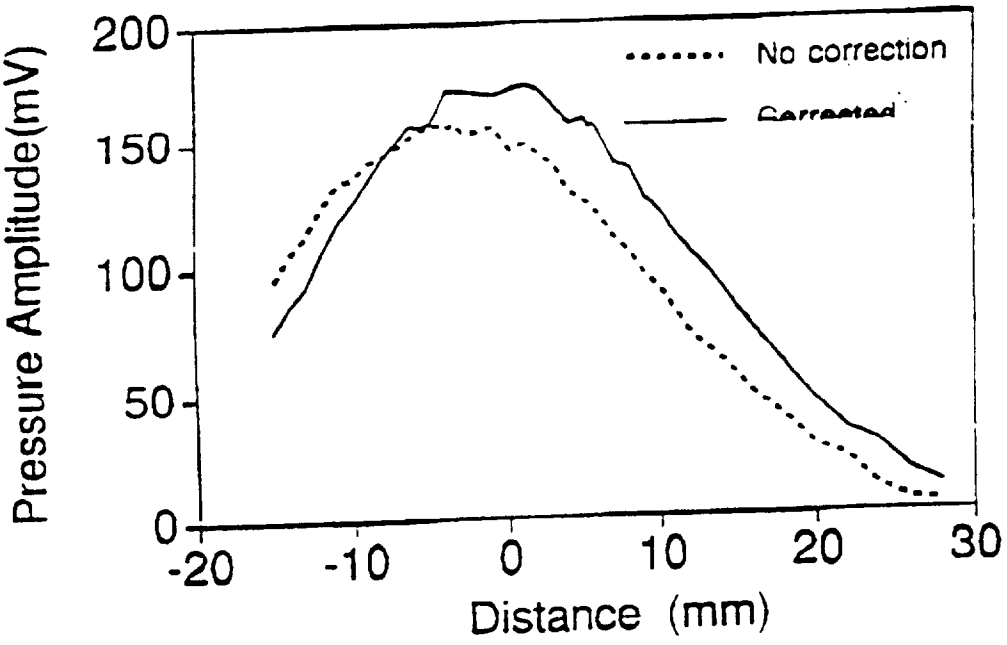

Pressure Amplitude(mV)
200
150
100
50
0
-20
-10
0
10
20
30
Distance (mm)
No correction
Corrected

Fig 5B

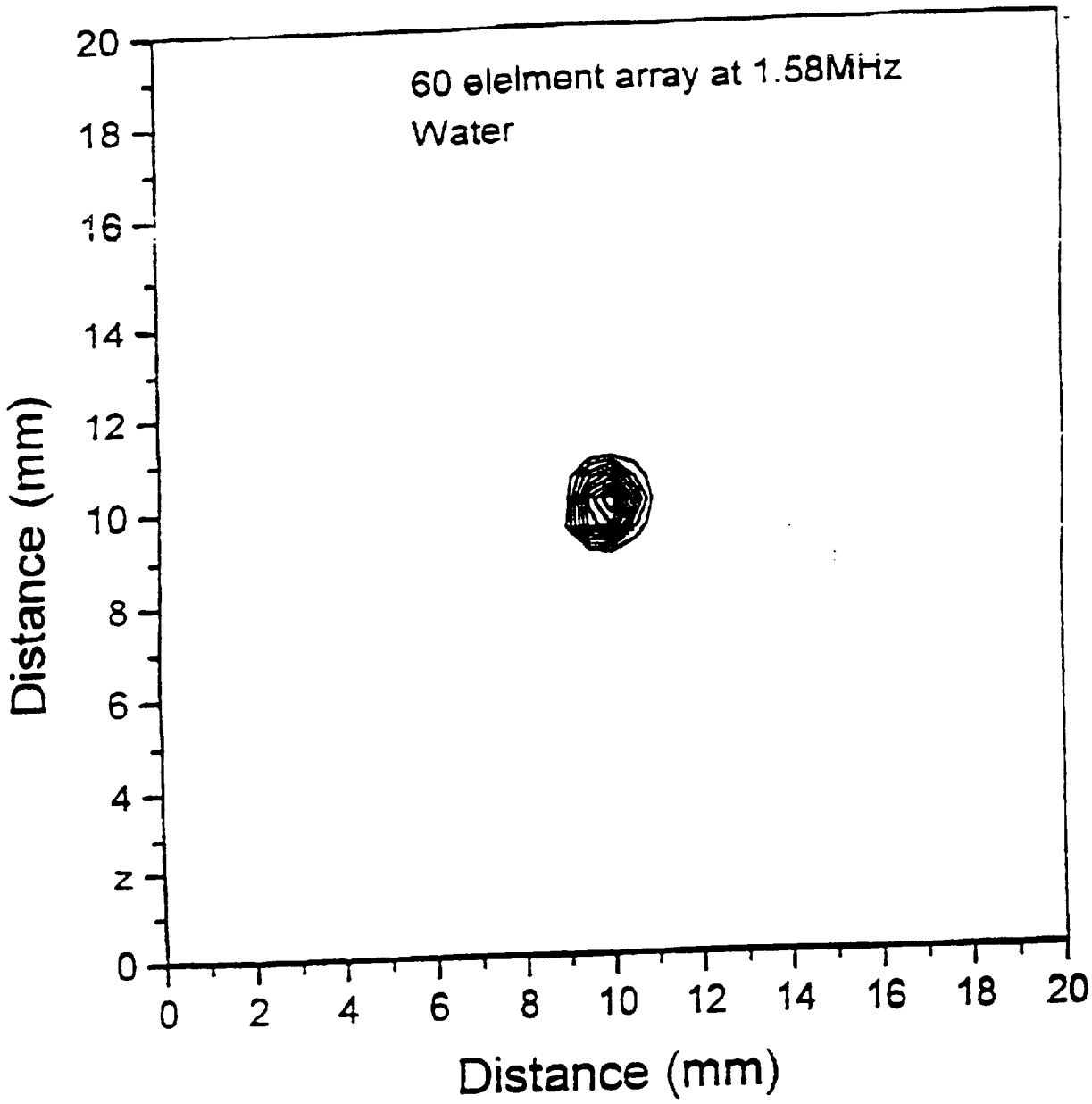
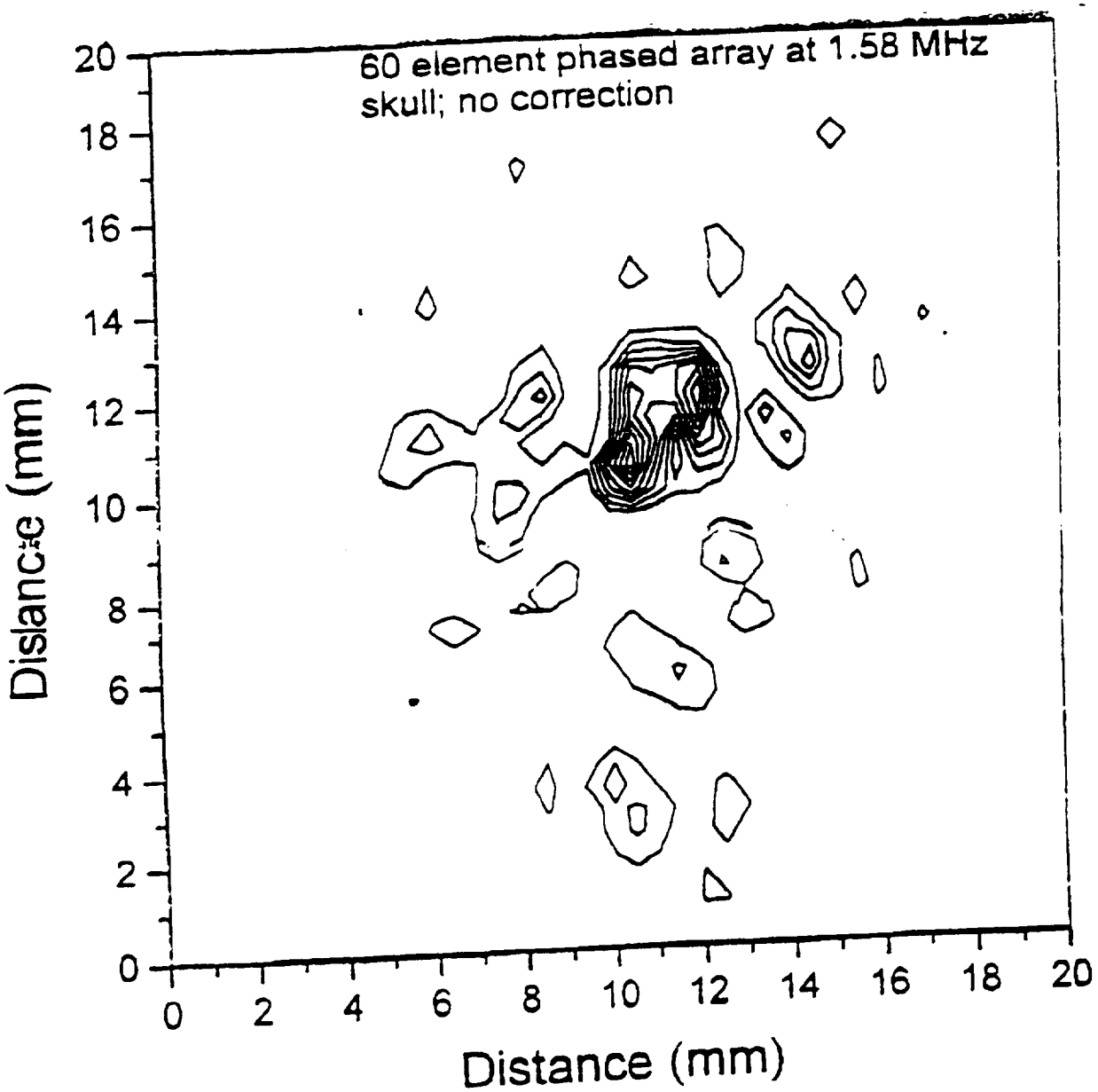
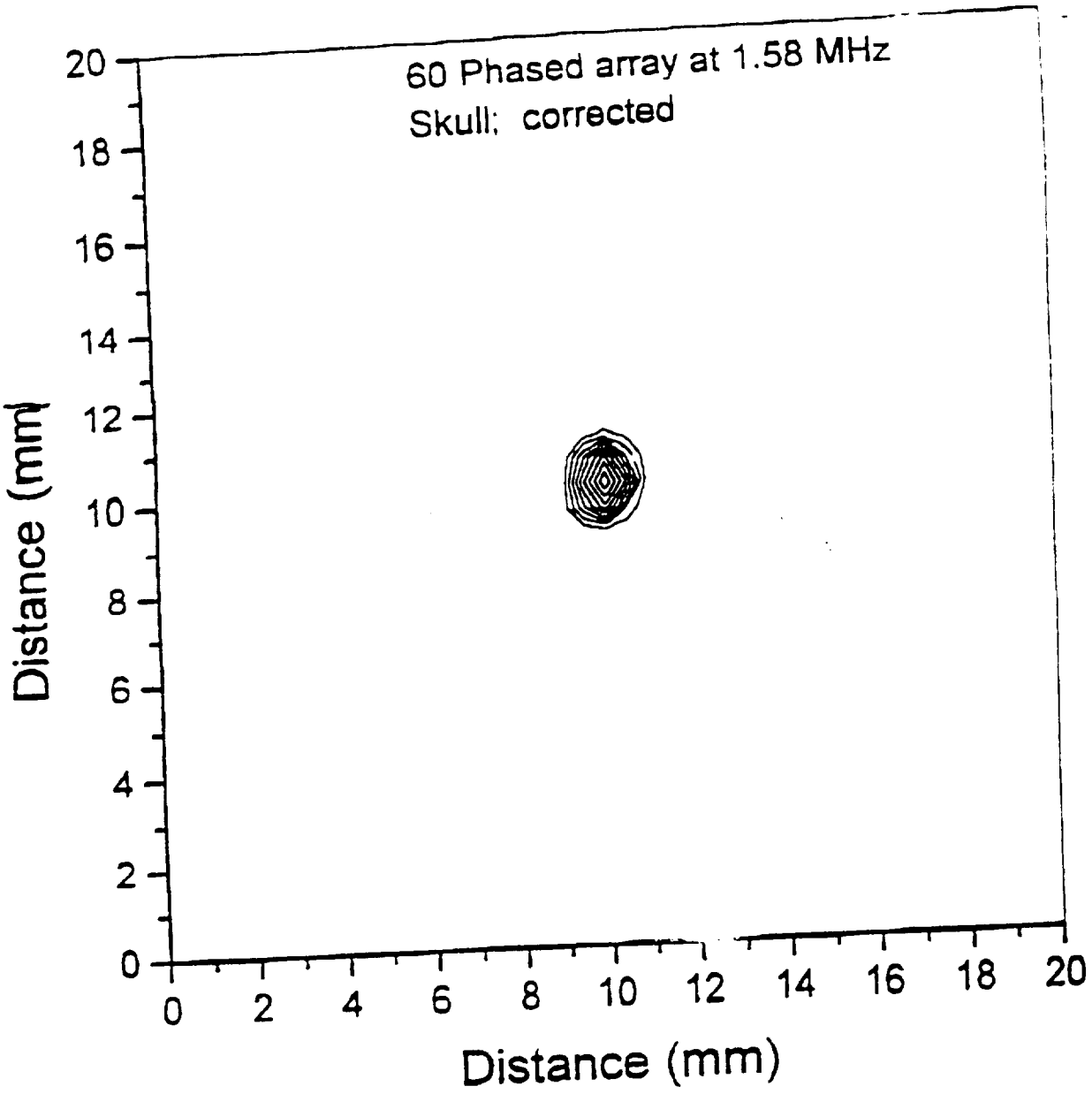

60 elelment array at 1.58MHz
Water
Distance (mm)
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20

Figure 5A


60 element phased array at 1.58 MHz
skull; no correction
Distance (mm)
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20

5B


60 Phased array at 1.58 MHz
Skull: corrected
Distance (mm)
Distance (mm)
0
2
4
6
8
10
12
14
16
18
20

S.C.

In view thereof, what we claim is:

1. A method for delivering a compound from the bloodstream to a selected location in the brain, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change, and

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location.

2. A method according to claim 1, wherein steps (A) and (C) include the step of delivering the ultrasound through the skull.

3. A method according to claim 2, wherein steps (A) and (C) include the step of delivering the ultrasound using any of (i) a phased array, (ii) a focused ultrasound transducer, and (iii) a combination of an ultrasound source and acoustic lens.

4. A method according to claim 1, wherein steps (A) and (C) include the steps of

exposing dura matter of the brain,

delivering the ultrasound substantially at or beneath the exposed dura matter.

5. A method according to claim 1, wherein step (B) includes the step of generating a radiologic image of at least a portion of the brain in the vicinity of the selected location.

6. A method according to claim 1, comprising admitting the compound into the patient's bloodstream at least in a vicinity of the selected location.

7. A method according to claim 1, wherein step (C) includes the step of delivering the ultrasound to the confirmed location in the brain to open the blood-brain barrier by cavitation.

8. A method according to claim 7, wherein step (C) includes the step of delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

9. A method according to claim 1, wherein step (C) includes the step of delivering the ultrasound to the confirmed location in the brain to open the blood-brain barrier by heating.

10. A method according to claim 9, wherein step (C) includes the step of delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

11. A method according to claim 1, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain to induce by cavitation the change detectable by imaging.

12. A method according to claim 11, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain at a frequency ranging from 20 kHz to 5 MHz, and with sonication duration ranging from 100 nanoseconds to 1 minute.

13. A method according to claim 1, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain to induce by heating the change detectable by imaging.

14. A method according to claim 13, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain at a frequency ranging from 200 kHz to 10 MHz, and with sonication duration ranging from 100 milliseconds to 30 minutes.

15. A method according to any of claims 11 - 14, wherein step (A) includes the steps of

introducing a contrast agent into the patient, at least in a vicinity of the selected location in the brain, and

effecting a change at that location detectable by imaging by applying ultrasound there to open the blood-brain barrier and, thereby, induce uptake of the contrast agent there.

16. A method according to claim 1, wherein step (C) includes the step of introducing into the patient's bloodstream a compound including any of a contrast agent, a neuropharmacologic agent, a neuroactive peptides, a protein, an enzyme, a gene therapy agent, a neuroprotective factor, a growth factor, a biogenic amine, a trophic factor to any of brain and spinal transplants, an immunoreactive proteins, a receptor binding protein, a radioactive agent, an antibody, and a cytotoxin.

17. A method for delivering a compound to a selected location in the brain of a patient, the method comprising

A. introducing a contrast agent into the patient's bloodstream, at least in a vicinity of the selected location in the brain,

B. applying ultrasound to the selected location to open the blood-brain barrier and, thereby, induce uptake of the contrast agent there.

C. taking a radiologic image of at least a portion of the brain to confirm the location to which ultrasound was delivered,

D. introducing the compound into the patient's bloodstream at least in a vicinity of the confirmed location, and

E. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound there.

18. An apparatus for delivering a compound to a selected location in the brain of a patient, the apparatus comprising

A. an ultrasound source,

B. targeting means, coupled to the ultrasound source, for applying ultrasound to the selected location in the brain to effect at that location a change detectable by imaging,

C. imaging means for generating a radiologic image of at least a portion of the brain to confirm the location of that change, and

D. delivery means, coupled to the ultrasound source, for applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound there.

19. An apparatus according to claim 18, wherein at least one of the targeting means and the delivery means includes means for delivering the ultrasound through the skull.

20. An apparatus according to claim 19, wherein at least one of the targeting means and the delivery means comprises any of (i) a phased array, (ii) a focused ultrasound transducer, and (iii) a combination of an ultrasound source and acoustic lens.

21. An apparatus according to claim 18, wherein at least one of the targeting means and the delivery means includes means for delivering the ultrasound substantially at or beneath exposed dura matter.

22. An apparatus according to claim 18, wherein the imaging means comprises means for generating a radiologic image of at least a portion of the brain in the vicinity of the selected location.

23. An apparatus according to claim 18, wherein the delivery means includes means for delivering the ultrasound to the confirmed location in the brain to open the blood-brain barrier by cavitation.

24. An apparatus according to claim 23, wherein the delivery means includes means for delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

25. An apparatus according to claim 18, wherein the delivery means includes means for delivering the ultrasound to the confirmed location in the brain to open the blood-brain barrier by heating.

26. An apparatus according to claim 25, wherein the delivery means includes means for delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

27. An apparatus according to claim 18, wherein the targeting means includes means for delivering the ultrasound to the selected location in the brain to effect radiologic activity by cavitation.

28. An apparatus according to claim 27, wherein the targeting means includes means for delivering the ultrasound to the selected location in the brain at a frequency ranging from 20 kHz to 5 MHz, and with sonication duration ranging from 100 nanoseconds to 1 minute.

29. An apparatus according to claim 18, wherein the targeting means includes means for delivering the ultrasound to the selected location in the brain to induce by heating the change detectable by imaging.

30. An apparatus according to claim 29, wherein the targeting means includes means for delivering the ultrasound to the selected location in the brain at a frequency ranging from 200 kHz to 10 MHz, and with sonication duration ranging from 100 milliseconds to 30 minutes.

31. An apparatus for delivering a compound to a selected location in the brain of a patient, the method comprising

A. an ultrasound source,

A. targeting means, coupled to the ultrasound source, for applying ultrasound to the selected location to open the blood-brain barrier and, thereby, induce uptake of the contrast agent there.

C. imaging means for taking a radiologic image of at least a portion of the brain to confirm the location to which ultrasound was delivered, and

D. delivery means, coupled to the ultrasound source, for applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound there.

32 A method for delivering a compound from the bloodstream to a selected location in the brain, the method comprising

A. applying ultrasound to a selected location in the brain to (i) induce a change at that location detectable by imaging, and (ii) open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location, and

B. generating a radiologic image of at least a portion in the brain to confirm the location of the change detectable by imaging.

33. A method according to claim 32, wherein step (A) includes the step of delivering the ultrasound through the skull.

34 A method according to claim 33, wherein step (A) includes the step of delivering the ultrasound using any of (i) a phased array, (ii) a

focused ultrasound transducer, and (iii) a combination of an ultrasound source and acoustic lens.

35. A method according to claim 32, wherein step (A) includes the steps of

exposing dura matter of the brain,

delivering the ultrasound substantially at or beneath the exposed dura matter.

36. A method according to claim 32, wherein step (B) includes the step of generating a radiologic image of at least a portion of the brain in the vicinity of the selected location.

37. A method according to claim 32, comprising admitting the compound into the patient's bloodstream at least in a vicinity of the selected location.

38. A method according to claim 32, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain to induce at that location a change detectable by imaging and to open the blood-brain barrier by cavitation.

39. A method according to claim 38, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain at a frequency ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

40. A method according to claim 32, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain to induce by heating the change detectable by imaging and to open the blood-brain barrier.

41. A method according to claim 40, wherein step (A) includes the step of delivering the ultrasound to the selected location in the brain at a frequency ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

42. A method according to claim 32, wherein step (A) includes the step of introducing into the patient's bloodstream a compound including any of a contrast agent, a neuropharmacologic agent, a neuroactive peptides, a protein, an enzyme, a gene therapy agent, a neuroprotective factor, a growth factor, a biogenic amine, a trophic factor to any of brain and spinal transplants, an immunoreactive proteins, a receptor binding protein, a radioactive agent, an antibody, and a cytotoxin.

43. An apparatus for delivering a compound to a selected location in the brain of a patient, the apparatus comprising

A. an ultrasound source,

B. targeting/delivery means, coupled to the ultrasound source, for applying ultrasound to the selected location in the brain to (i) effect a change at that location detectable by imaging, and (ii) open the blood-brain barrier at that location and, thereby, induce uptake of the compound there, and

C. imaging means for generating a radiologic image of at least a portion of the brain to confirm the location of the change detectable by imaging.

44. An apparatus according to claim 43, wherein targeting/deliver means includes means for delivering the ultrasound through the skull.

45. An apparatus according to claim 44, wherein targeting/deliver means comprises any of a (i) a phased array, (ii) a focused ultrasound transducer, and (iii) a combination of an ultrasound source and acoustic lens.

46. An apparatus according to claim 43, wherein targeting/deliver means includes means for delivering the ultrasound substantially at or beneath exposed dura matter.

47. An apparatus according to claim 43, wherein the imaging means comprises means for generating a radiologic image of at least a portion of the brain in the vicinity of the selected location.

48. An apparatus according to claim 43, wherein the targeting/deliver means includes means for delivering the ultrasound to the selected location in the brain to induce by cavitation a change detectable by imaging and to open the blood-brain barrier.

49. An apparatus according to claim 48, wherein the targeting/delivery means includes means for delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 20 kHz to 10 MHz, sonication duration ranging from 100 nanoseconds to 30 minutes, with continuous wave or burst mode operation, where the burst mode repetition varies from 0.01 Hz to 1 MHz.

50. An apparatus according to claim 43, wherein the targeting/delivery means includes means for delivering the ultrasound to the confirmed location in the brain to induce by heating the change detectable by imaging and to open the blood-brain barrier by heating.

51. An apparatus according to claim 50, wherein the targeting/delivery means includes means for delivering the ultrasound to the confirmed location in the brain at a frequency ranging from 250 kHz to 10 MHz, and with sonication duration ranging from 0.10 microseconds to 30 minutes.

52. A method for delivering a compound from the bloodstream to a selected location in the brain of a patient, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change,

C. further confirming that location by a change in central nervous system function of the patient,

D. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location.

53. A method for delivering a compound from the bloodstream to a selected location in the brain of a patient, the method comprising

A. applying ultrasound to a selected location in the brain to effect a change in a central nervous system function of the patient,

B. confirming that location by a change in that central nervous system function, and

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location.

54 A method for delivering a compound from the bloodstream to a selected location in the brain, the method comprising

A. applying ultrasound to a selected location in the brain to (i) induce a change at that location detectable by imaging, and (ii) open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location,

B. generating a radiologic image of at least a portion in the brain to confirm the location of the change detectable by imaging, and

C. further confirming that location by a change in central nervous system function of the patient.

55 A method for delivering a compound from the bloodstream to a selected location in the brain of a patient, the method comprising

A. applying ultrasound to a selected location in the brain to (i) a change in a central nervous system function of the patient, and (ii) open the blood-brain barrier at that location and, thereby, induce uptake of the compound at that location, and

B. confirming that location by detecting a change in that central nervous system function.

56. A method for treating at least one of a neurological and a neurologically-related disorder, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change,

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake at that location of a compound in the bloodstream.

57. A method for treating at least one of a neurological and a neurologically-related disorder, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change,

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake at that location of a compound in the bloodstream.

58. A method for modification of neurologic and neurologically-related activity, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change,

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake at that location of a compound in the bloodstream.

59. A method for functional neurosurgery, the method comprising

A. applying ultrasound to a selected location in the brain to effect at that location a change detectable by imaging,

B. generating a radiologic image of at least a portion in the brain to confirm the location of that change,

C. applying ultrasound to the confirmed location in the brain, or a location based thereon, to open the blood-brain barrier at that location and, thereby, induce uptake at that location of at least a selective cytotoxin in the bloodstream.

FIG. 1

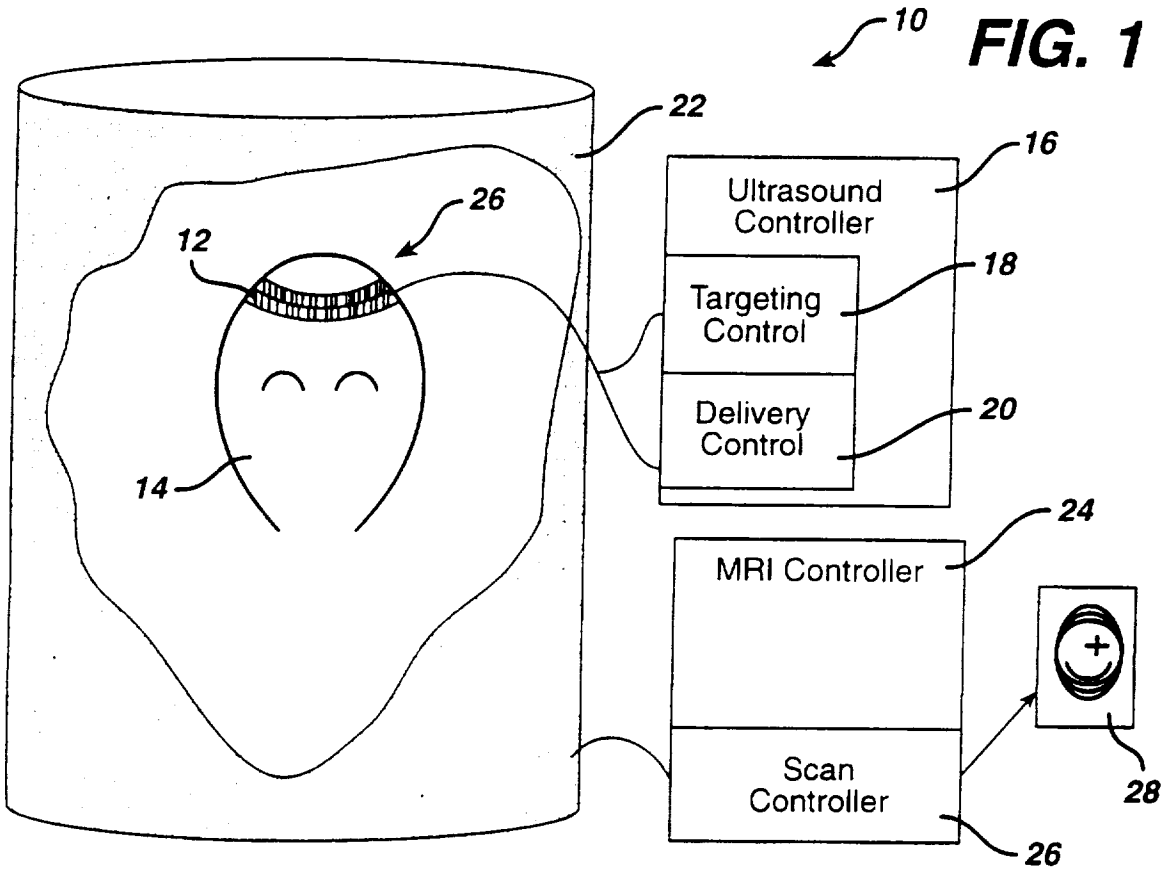
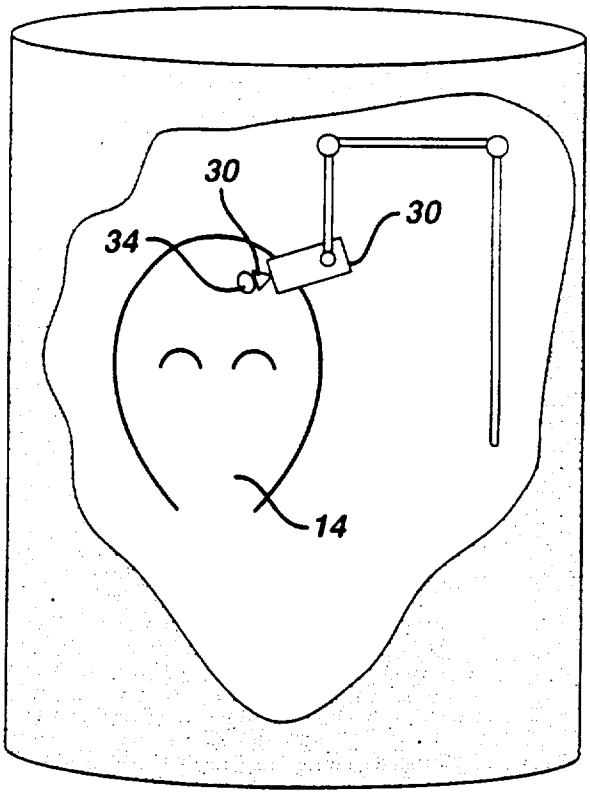

10
22
16
Ultrasound Controller
26
12
18
Targeting Control
20
Delivery Control
14
24
MRI Controller
28
Scan Controller
26

FIG. 2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30
34
14

FIG. 3

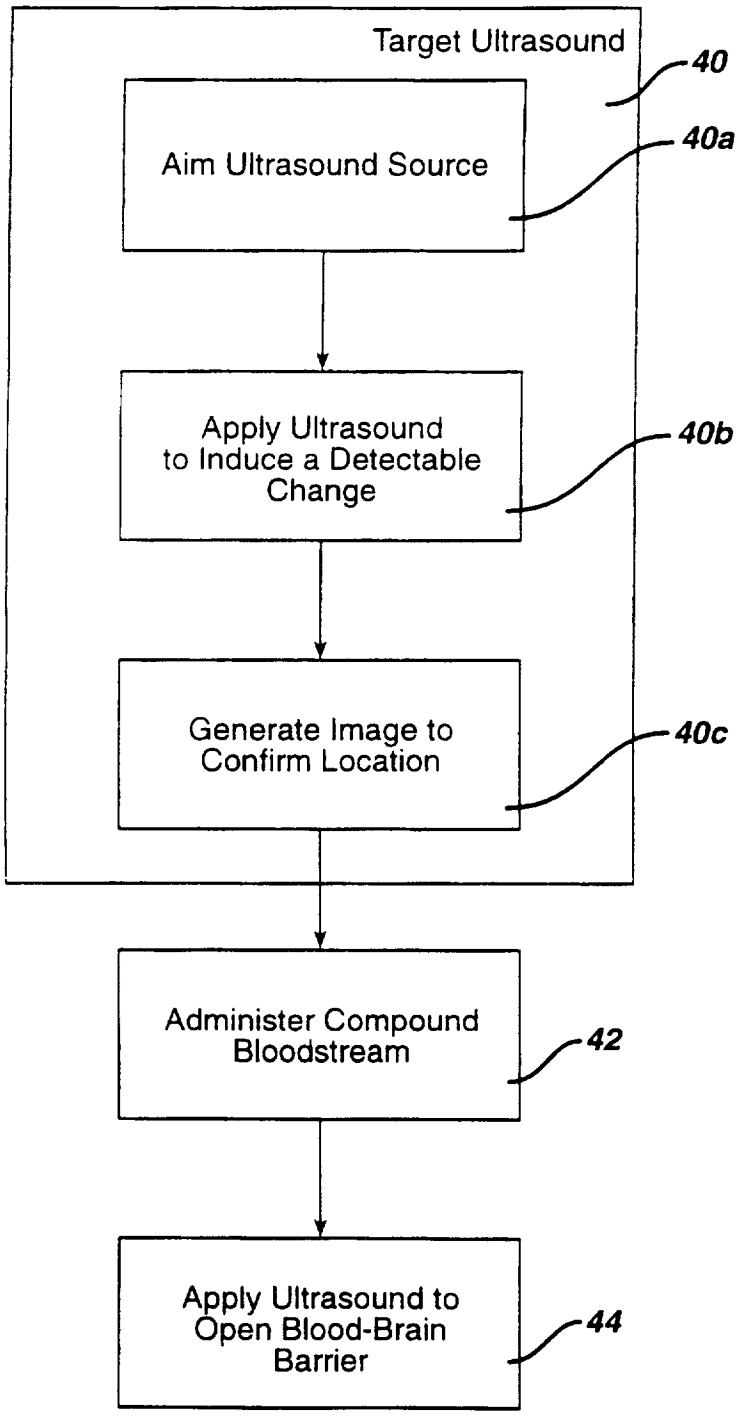

Target Ultrasound
40
Aim Ultrasound Source
40a
Apply Ultrasound to Induce a Detectable Change
40b
Generate Image to Confirm Location
40c
Administer Compound Bloodstream
42
Apply Ultrasound to Open Blood-Brain Barrier
44

FIG. 4

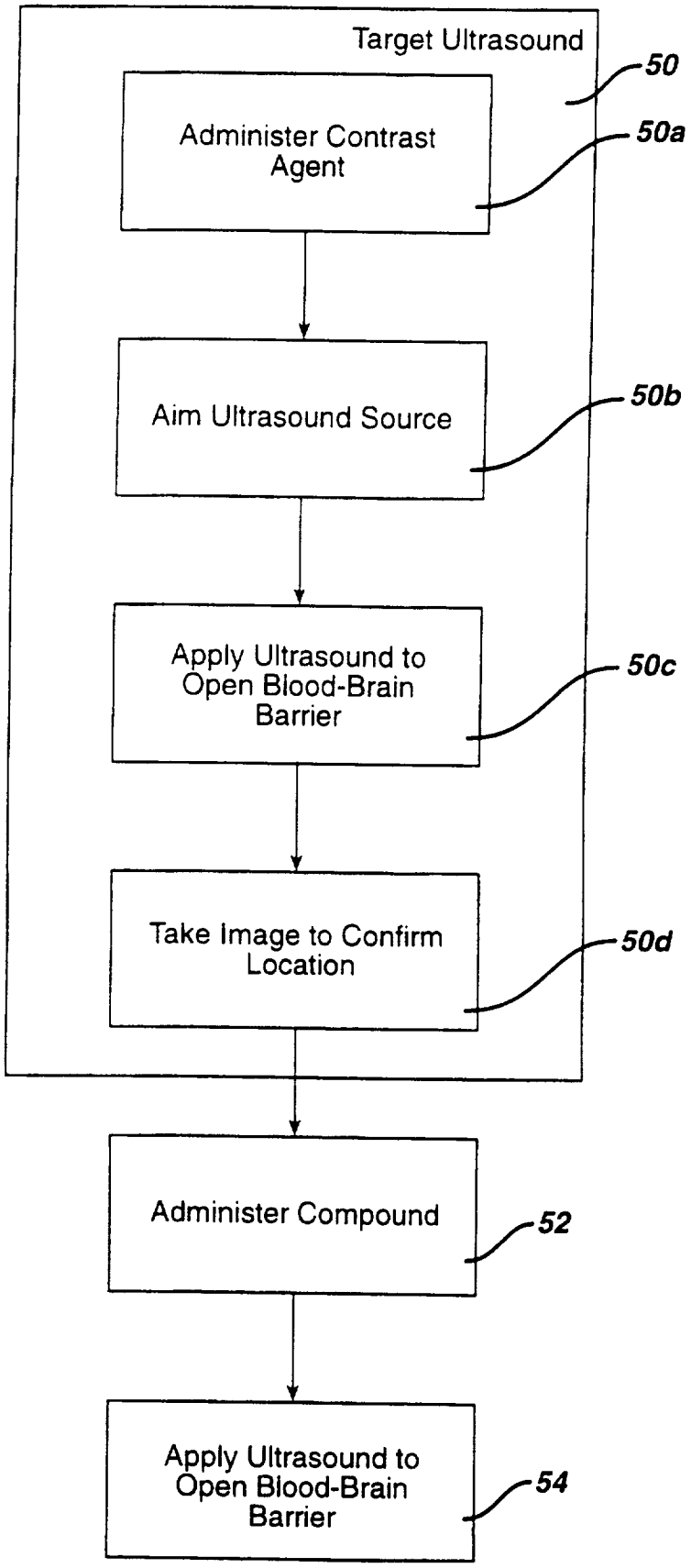

Target Ultrasound
50
Administer Contrast Agent
50a
Aim Ultrasound Source
50b
Apply Ultrasound to Open Blood-Brain Barrier
50c
Take Image to Confirm Location
50d
Administer Compound
52
Apply Ultrasound to Open Blood-Brain Barrier
54

FIG. 5

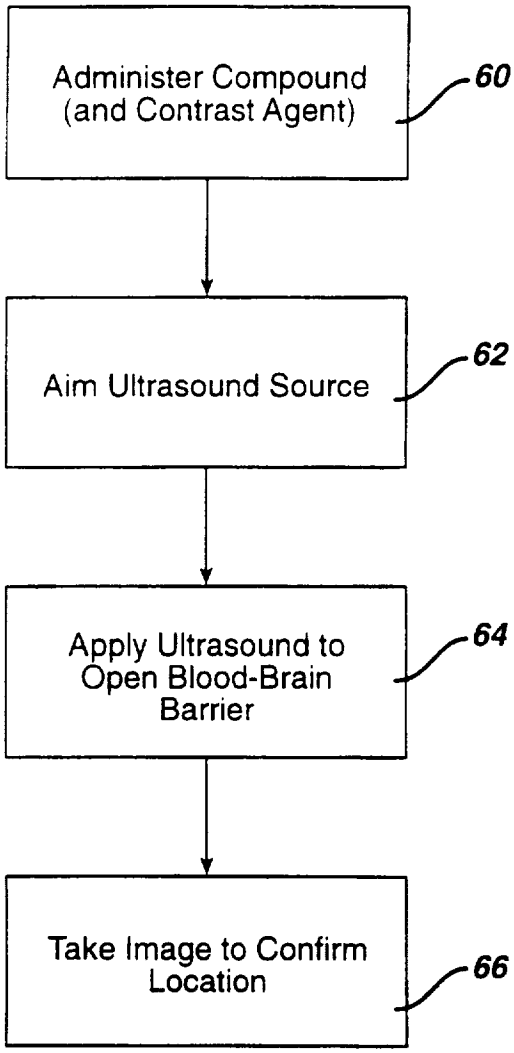
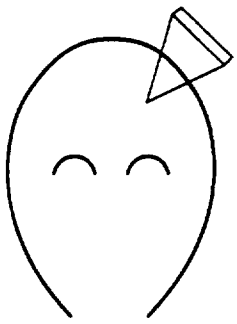

Administer Compound (and Contrast Agent)
60
Aim Ultrasound Source
62
Apply Ultrasound to Open Blood-Brain Barrier
64
Take Image to Confirm Location
66

FIG. 6A



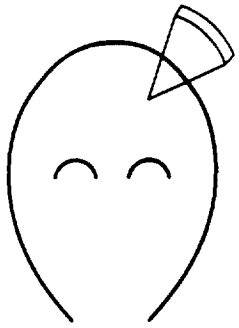
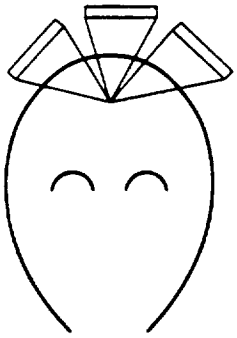


FIG. 6B

FIG. 6C



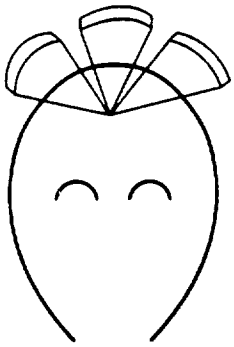
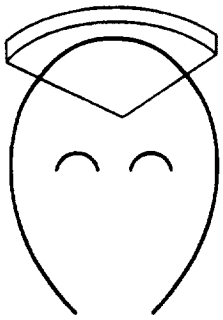


FIG. 6D

FIG. 6E



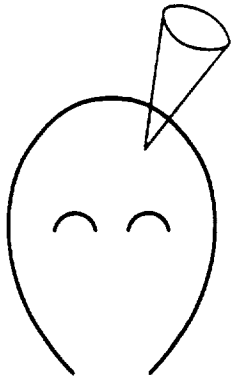
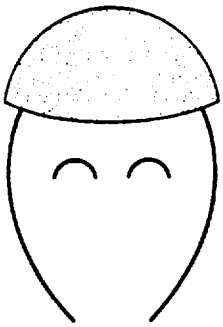


FIG. 6F

FIG. 6G



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14737

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61B 05/00, 17/00
US CL :128/653.1, 660.03

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/653.1, 653.2, 653.4, 654, 660.03, 897, 898; 424/9.3; 601/002, 004

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS
Search Terms: ultrasound and blood brain barrier

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,923,437 A (GORDON) 08 MAY 1990, ABSTRACT, FIG. 2, COL. 1 LINES 54-68, AND COL. 2 LINES 44-59.	1-59
A	US 5,465,718 A (HOCHMAN ET AL) 14 NOVEMBER 1995, ABSTRACT, AND CLAIM 1.	1-59
A	US 5,438,989 A (HOCHMAN ET AL) 08 AUGUST 1995, ABSTRACT, AND CLAIMS 2 AND 6.	1-59
A	US 5,112,596 A (MALFROY-CAMINE) 12 MAY 1992, ABSTRACT, AND COL. 1 LINES 1-16 AND 28-37.	1-59
A	US 5,059,415 A (NEUWELT) 11 OCTOBER 1991, ABSTRACT, AND CLAIMS 1 AND 6.	1-59
A	US 4,303,636 A (GORDON) 01 DECEMBER 1981, ABSTRACT, AND COL. 2 LINES 1-56.	1-59

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
18 OCTOBER 1997

Date of mailing of the international search report
12 NOV 1997

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
SHAWNA SHAW
Telephone No. (703) 308-2985

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/14737

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,315,514 A (DREWES et al) 16 February 1982, Abstract, and Fig. 1.	1-59
A	US 3,499,437 A (BALAMUTH) 10 March 1970, Figs. 1, 3, 9, 10 and 14, col. 4 lines 70-75, and claim 1.	1-59
A	US 5,307,816 A (HASHIMOTO et al) 03 May 1994, Abstract, Fig. 1, and claim 1.	1-59
A	US 5,443,068 A (CLINE et al) 22 August 1995, Abstract, and Fig. 1.	1-59
A	US 5,524,620 A (ROSENSCHEIN) 11 June 1996, Abstract, and Figs. 1 and 3.	1-59
A	US 5,485,839 A (AIDA et al) 23 January 1996, Abstract, and Figs. 1-3.	1-59
A	US 5,526,814 A (CLINE et al.) 18 June 1996, Abstract, and Fig. 1.	1-59
A	US 5,368,032 A (CLINE et al) 29 November 1994, Abstract, Figs. 1 and 2, and claim 1.	1-59
A	US 5,291,890 A (CLINE et al) 08 March 1994, Abstract, and claim 1.	1-59