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REVIEW ARTICLE

Microbubble-enhanced Focused Ultrasound-induced Blood—brain Barrier Opening for Local and Transient Drug Delivery in Central Nervous System Disease

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The blood—brain barrier is a specialized protective structure in the central nervous system, which is critical for maintaining brain homeostasis and low permeability to control the passage of molecules from the circulation into the brain parenchyma and the efflux from the brain. However, the blood—brain barrier also hinders the transportation of therapeutic agents and contrast agents from the blood into brain tissue, lowering treatment efficiency. Recently, focused ultrasound sonication with microbubbles has been proved to transiently open the blood—brain barrier, allowing the penetration of administered agents or drugs into the brain. In this article, we review the current state of this drug delivery technique, its application in preclinical brain disease models, and treatment planning for this novel technique.

Blood—brain barrier

Concept of the blood—brain barrier

The blood—brain barrier (BBB) was first discovered by Paul Ehrlich [1]. He noted that when he administrated a dye into an *in vivo* circulatory system, all the organs were stained except for the brain and the spinal cord. Further evidence of the BBB was provided by Max Lewandowsky [2] and Edwin Goldmann [3]. Lewandowsky [2] discovered the limited

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permeability of potassium ferrocyanate into the brain, and Goldmann [3] noticed that when he directly injected trypan blue dye into the cerebrospinal fluid, only cells within the brain were stained. Although these findings offered indications for the presence of the BBB, the concept of the BBB was confirmed by Davson and Spaziani [4], who demonstrated that cerebral capillaries prevent the diffusion of sucrose, iodide, and p-aminohippurate into the brain. In 1967, Reese and Karnovsky [5] designed further experiments to visualize the BBB using horseradish peroxidase.

Structure of the BBB

The capillary network within the brain is extremely packed (about 20 m^2/1300 g in human brain [6]) and is intricate. Thus, each neuron is perfused by its own microvasculature [7]. The BBB is a specialized substructure within the central nervous system (CNS) blood vascular system, which consists of endothelial cells (ECs) connected together by tight junctions (TJs), basement membranes, pericytes, and astrocytes [8]. This layered structure acts as the brain's frontline defense against toxic and harmful materials in the blood stream. The TJs are located in the cerebrovascular endothelium, which contains membrane-associated guanylate kinases such as cingulin, occludin, and the cadhedrins (single-pass membrane-spanning molecules) ZO-1 and ZO-2 [9]. The presence of TJs prevents circulating substances from entering the brain via paracellular routes, although they can reach the brain through the ECs of brain microvessels via a transcellular pathway or via specialized receptor-mediated transcytosis and transport proteins [10]. The basement membrane supports the abluminal surface of the astrocytes, ECs, and pericytes [11]. The combination of ECs, astrocyte feet, and pericytes makes the BBB less permeable to large-molecular-weight (>500 Da) [12], water-soluble, and ionic substances [13]. Therefore, the BBB prevents nearly 100% of macromolecule therapeutic drugs and diagnostic substances, and about 98% of small-molecule agents from penetrating the brain, which is a great disadvantage for the treatment of CNS disease [14].

Methods for increasing BBB permeability

There are a number of preclinical and clinical approaches for crossing the BBB to enhance drug delivery into the brain tissue, including: (1) chemical modification of drugs to make them lipophilic, (2) use of drug carriers to transport drugs across the BBB, (3) intravenous (i.v.) administration of hypertonic solutions to open the BBB, and (4) direct transcranial injection of drugs through a needle or catheter to bypass the BBB structure and reach the brain. In approach (1), the drug is modified with lipid-soluble functional groups (e.g., amino acids) or conjugated to lipid carriers (e.g., free fatty acid, adamantane, or dihyropyridine) to achieve drug lipidization. However, the main problem with drug lipidization lies in the increased uptake of the lipidized drug by peripheral organs, reducing the drug concentration in the brain tumor [7]. In approach (2), the drug is encapsulated into carriers (e.g., liposomes or nanoparticles [15]), or conjugated to proteins [16], peptide vectors [17], or antibodies [18]. These drug carrier devices then promote the transportation of the drug across the BBB using a BBB endogenous-carrier system and/or a receptor-mediated transcytosis route. Due to the finite amount of receptors *in vivo* and the limited payload of a drug carrier, the drug carrier approach is limited by inadequate transportation of the drug. In approach (3), the infusion of hyperosmotic solution (e.g., mannitol and arabinose) induces shrinkage of the brain and brain capillary ECs, and thus leads to a transient opening of the TJs [19]. However, this method induces nonlocalized drug delivery as well as promotes the penetration of toxic substance into the brain tissues (e.g., plasma albumin or other blood protein components), thereby causing damage to surrounding normal brain cells and neural cells [20]. In approach (4), the drug is directly delivered to the brain via either an intracerebroventricular injection or an intracerebral implantation [21]. Nevertheless, the drug can only penetrate the brain tissue via diffusion, which makes it difficult to cover the whole lesion from the depot site. Further, this method may produce invasive traumas during the injection process.

Therapeutic effect of focused ultrasound

The piezoelectric materials of an ultrasound probe can be manufactured into an arc shape, or electric phase modulation can be used to focus transmitted ultrasound energy, thus enabling focused ultrasound (FUS). FUS allows noninvasive accumulation of acoustic energy within a focal spot inside the body, with negligible biological effects to the surrounding tissues and near-fields. There are two kinds of mechanisms for inducing biological effects by FUS, thermal and nonthermal (e.g., cavitation), both of which are discussed below.

When exposing biological tissues to FUS for long durations, the acoustic energy is attenuated and absorbed by the surrounding tissues, and then converted into thermal energy, producing a regional temperature rise. This temperature-rising phenomenon has been shown to persist for minutes to hours, and is further introduced in treatment applications, called hyperthermia. However, previous studies have indicated that temperatures in the range of 43—46°C have detrimental effects on the brain tissue such as ammonia production, hemiparesis, and even death [22]. By contrast, tumor cells are believed to be particularly sensitive to heat, and therefore, FUS-induced hyperthermia (at 43°C for 30—60 minutes) can be used to increase the sensitivity of tumors to other interventions (radiation therapy, chemotherapy, and immunotherapy) used to treat cancers [23]. At higher temperatures (>60°C), FUS is also applied as a thermal ablation procedure to defeat solid tumors. Many studies have demonstrated the feasibility of using thermal ablation to remedy a variety of tumors, including kidney, uterus, liver, breast, bone, pancreas, and prostate [24,25]. In addition, thermal coagulation of blood vessels has also been proposed as a medical application of FUS [26,27].

One major limitation of ultrasound-induced thermal therapy in the brain is the strong absorption and attenuation of acoustic energy by the skull. Therefore, for clinical brain therapeutic application, an invasive craniotomy is