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Pharmacokinetics of doxorubicin in glioblastoma multiforme following ultrasound-Induced blood-brain barrier disruption as determined by microdialysis

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ABSTRACT

The goal of this study was to investigate the *in vivo* extracellular kinetics of doxorubicin (Dox) in glioblastoma multiforme (GBM)-bearing mice following focused ultrasound (FUS)-induced blood-brain barrier (BBB) disruption using microdialysis. An intracranial brain tumor model in NOD-*scid* mice using human brain GBM 8401 cells was used in this study. Prior to each sonication, simultaneous intravenous administration of Dox and microbubbles, and the Dox concentration in the brains was quantified by high performance liquid chromatography (HPLC). Drug administration with sonication elevated the tumor-to-normal brain Dox ratio of the target tumors by about 2.35-fold compared with the control tumors. The mean peak concentration of Dox in the sonicated GBM dialysate was 10 times greater than without sonication, and the area under the concentration-time curve was 3.3 times greater. This study demonstrates that intracerebral microdialysis is an effective means of evaluating real-time target BBB transport profiles and offers the possibility of investigating the pharmacokinetics of drug delivery in the sonicated brain.

1. Introduction

The tight junctions of the blood-brain barrier (BBB) restrict the transport of hydrophilic drugs [1]. Altering the permeability of the BBB via the application of certain experimental methods, however, may change the delivery of drugs into the brain [2]. It has been shown that focused ultrasound (FUS) with microbubbles may provide a noninvasive tool for local drug delivery in the brain due to FUS-induced BBB disruption [3]. Use of this FUS technology has been shown to not only significantly increase the accumulation of drugs at the sonicated tumor but also to significantly elevate the tumor-to-normal brain ratio in the focal region [4,5]. Moreover, the antitumor effects of chemotherapeutic drugs on brain tumors can be enhanced following FUS exposure [6].

Liposome-encapsulated chemotherapy was designed to increase the selectivity of drugs in tumors as compared to normal tissue. For example, liposomal doxorubicin (Lipo-Dox) was developed to enhance the delivery of Dox to brain tumors [7]. By binding isotopes to the drugs, the pharmacokinetics of nuclear imaging can be evaluated in a living animal model at several time points. Our previous work has demonstrated that ^{111}In-liposomes micro-SPECT/CT should be able to provide important information about the optimum therapeutic window for brain tumor chemotherapy following FUS exposure [8]. However, the nuclear imaging provided by this method comes from the total radioactivity in a given tissue, such that no free drug concentrations can be measured appropriately. Thus, a monitoring method is required that directly measures the concentration of a drug in the brain in order to assess the pharmacokinetic efficacy of brain delivery systems.

Microdialysis is an important sampling tool in many pharmacokinetics [9]. Intracerebral microdialysis involves the insertion of a probe into the selected region of the brain. Drug concentrations in brain dialysate reflect concentrations in brain extracellular fluid (ECF). To determine the transport characteristics of drug across the BBB, the free drug concentrations in blood should be detected

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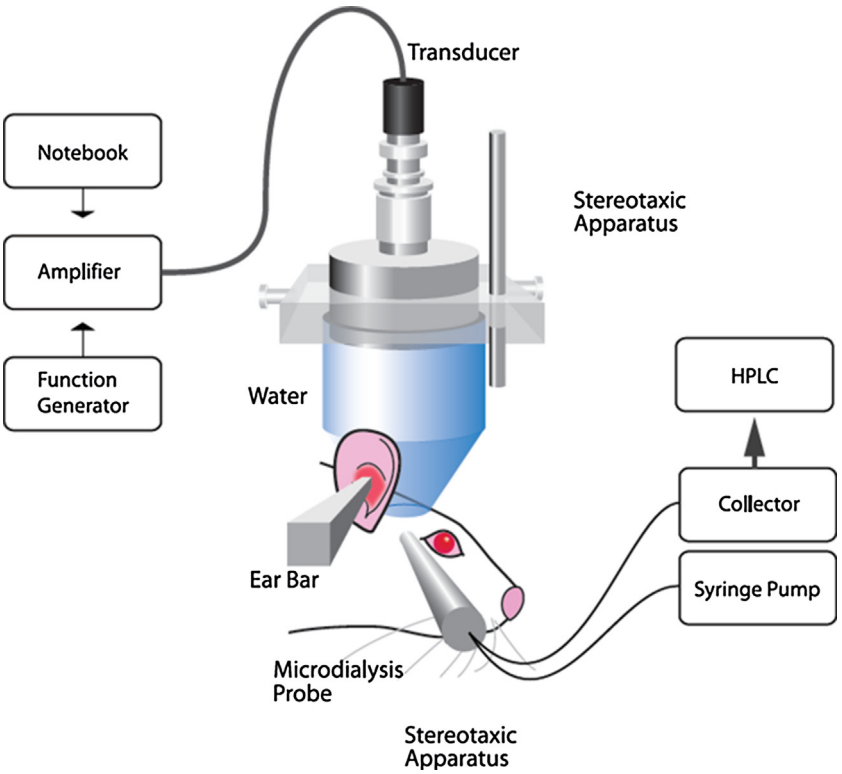

Fig. 1. Schematic diagram of the experimental setup for FUS drug delivery monitored by microdialysis.

in parallel to brain ECF concentrations [10]. A previous study has demonstrated that microdialysis is a useful tool for assessing local BBB transport profiles of boronophenylalanine-fructose (BPA-f) in glioma-bearing rats with FUS-induced BBB disruption [11]. Although microdialysis is a useful sampling technique, the sampling lipophilic compounds generally induce low extraction efficiencies [12]. The results of one study indicated that while Dox may be sampled using microdialysis, the results must be interpreted carefully [13].

In this study, microdialysis sampling was applied to Dox. The goal of this study was to investigate the concentration-time profile of Dox and its pharmacokinetics in brain tumors with FUS-induced BBB disruption after intravenous injection of Dox.

2. Materials and methods

2.1. Glioma xenograft animal model

Male NOD-*scid* mice (aged 6–8 weeks old) were anesthetized via intraperitoneal administration of a 40 mg/kg of body weight dose of pentobarbital. The mice were shaved on the head above the nape of the neck, scrubbed with betadine/alcohol, and immobilized in a Cunningham Mouse/Neonatal Rat Adaptor stereotactic apparatus (Stoelting, Wood Dale, IL, USA). A 5-mm skin incision was made along the sagittal suture, and a burr hole was drilled into the skull. Human brain malignant glioma cells (GBM8401) were obtained from the Bioresource Collection and Research Center of Taiwan. 2×10^5 GBM8401 cells in 2 μL culture medium were injected into the brains of the mice. The glioma cells were stereotactically injected into a single location in the left hemisphere (0.14 mm anterior and 2.0 mm lateral to the bregma) of each mouse at a depth of 3.5 mm from the brain surface. Next, the burr holes in the skull were sealed with bone wax and the wound was flushed with iodinated alcohol. The progress of tumor growth was monitor by MR imaging. All animal experiments were performed according to the guidelines of and were approved by the Animal Care and Use Committee of National Yang-Ming University.

2.2. Pulsed ultrasound system

The pulsed-FUS exposures were generated by a 1.0 MHz single-element focused transducer (A392S, Panametrics, Waltham, MA, USA). The focal zone of the transducer was in the shape of an elongated ellipsoid, with a radial diameter (−6 dB) of 3 mm and an axial length (−6 dB) of 26 mm. The FUS system setup was detailed in our previous report [14]. A function generator (33220A, Agilent Inc., Palo Alto, USA) was connected to a power amplifier (500-009, Advanced Surgical Systems, Tucson, AZ) to create the US excitation signal. A power meter/sensor module (Bird 4421, Ohio, USA) was used to measure the input electrical power. The animals were anesthetized intraperitoneally with urethane (1.2 g/kg) prior to the experiments. Ultrasound contrast agent (UCA, SonoVue, Bracco International, Amsterdam, The Netherlands) was injected into the tail vein of each mouse approximately 15 s before each sonication. The transducer was applied with a duty cycle of 5% and a repetition frequency of 1 Hz. Each sonication time was 60 s at an acoustic power of 2.86 W and a UCA dose of 300 μL/kg. Ultrasound exposure was delivered to the tumor site on day 10 after the implantation of the tumor cells.

2.3. Microdialysis procedure

The entire experimental system was established to assess the variability of the Dox concentration in the brain tumor (Fig. 1). A commercially available microdialysis probe (CMA 12, membrane diameter: 0.5 mm; Stockholm, Sweden) was applied to sample the free Dox in the brain of each mouse. The given mouse was mounted on a stereotaxic frame for brain ECF sampling. An incision was made in the scalp, and a small hole was drilled on the left side of the skull for implantation of the rigid microdialysis probe in the