35th Annual International Conference of the IEEE EMBS
Osaka, Japan, 3 - 7 July, 2013

Chemotherapy of Glioblastoma by Targeted Liposomal Platinum Compounds with Focused Ultrasound

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Abstract—**Giloblastoma multiforme (GBM) is the most aggressive brain neoplasm, and patients have a poor prognosis after radiation and chemotherapy. The chemotherapy protocols still marginally improve the anti-tumor effect of patients with glioblastoma because the therapeutic dosage of many drugs is impeded by the blood–brain barrier (BBB). The use of liposomal drugs to GBM treatment might benefit from a more crossing of the BBB due to the lipid nature achieving higher doses of drug at the tumor sites. Human GBM-bearing mice were injected intravenously with cisplatin encapsulated in atherosclerotic plaque-specific peptide-1 (AP-1)-conjugated liposomes or unconjugated liposome. Moreover, the administration of AP-1 liposomal cisplatin (lipoplatin) followed by focused ultrasound (FUS)-induced BBB disruption. Tumor progression was monitored by biophotonic imaging. The preliminary data demonstrated that the GBM chemotherapy with AP-1 lipoplatin followed by pulsed FUS showed a modest improvement of tumor growth in the brain compared to the group treated with lipoplatin alone. Further investigations are needed to use this new targeted lipoplatin in treatment of malignancies.**

I. Introduction

Glioblastoma multiforme (GBM) is a high-grade, aggressive brain tumor, taking the lives of patients within 12 to 14 months after diagnosis [1-2]. It is hard to treat GBM completely by surgical resection due to diffuse infiltrative growth of the brain parenchyma. Radiation therapy remains the most effective treatment modality, chemotherapy have a slight survival benefit of GBM patients because the penetration of therapeutic agents are limited by the blood-brain barrier (BBB) [3]. Additionally, most tumors recur locally within 2 cm of the original location [4]. Recently, local and transient BBB disruption can be induced by focused ultrasound (FUS) combined with microbubbles [5-6]. FUS not only significantly promotes the accumulation of the agents at the sonicated site but also significantly increases the lesion-to-normal brain drug ratio in the focal location [7-8]. In addition, the duration and degree of BBB disruption can be regulated by acoustic parameters and the concentrations of microbubbles [9-10].

Cisplatin was being quite important and as one of the most effective therapeutic agents but it did cause nephrotoxicity [11-12]. The liposomal encapsulation of cisplatin (lipoplatin) had shown to produce similar efficacy to that of cisplatin and reduced all the toxicities of cisplatin. New types of antitumor agents can precisely target the biomarker of the malignant tumor cells, thus reducing the toxic effects in normal tissues. It has been indicated that human GBM cells express high levels of plasma membrane interleukin-4 receptors [13]. The human atherosclerotic plaque-specific peptide-1 (AP-1) peptides can locate atherosclerotic plaque tissue and bind to the IL-4 receptor, since it has the same binding motif to the IL-4 protein. FUS in conjunction with AP-1 conjugated liposomal doxorubicin could improve anti-tumor effects and reduce severe drug systemic toxicity [13-14]. Furthermore, combining FUS with drugs significantly elevated the tumor-to-normal brain drug ratio of the sonicated tumors compared to the control tumors [15].

The goal of this study was to investigate if the synergistic effect of target drug and FUS could improve the efficacy of chemotherapy for malignant brain tumors.

II. Methodology

A. *Glioblastoma Multiforme Model*

Male NOD-*scid* mice were anesthetized by an intraperitoneal administration of pentobarbital at a dose of 40 mg/kg of body weight. All procedures were performed according to guidelines and approved by the Animal Care and Use Committee of the National Yang-Ming University. Mice were shaved on the head above the nape of the neck, scrubbed with betadine/alcohol, and immobilized in a Cunning-ham Mouse/Neonatal Rat Adaptor stereotactic apparatus (Stoelting, Wood Dale, IL, USA). A 5-mm skin incision was made along the sagital suture and a burr hole drilled into the skull. Human brain malignant glioma cells (GBM8401) were obtained from the Bioresource Collection and Research Center of Taiwan [16]. GBM8401 cells were transformed with Luciferase gene (GBM8401-luc) and then 2×10^5 GBM8401-luc cells in 2 μL culture medium were injected into the brains of these mice. The glioma cells were stereotactically injected into the left hemisphere (0.14 mm anterior and 2.5 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. Tumor progression was monitored by bioluminescence imaging.

B. *Focused Ultrasound System*

The experimental set-up is presented in Fig. 1. Pulsed FUS was generated by a 1.0-MHz, single-element focused transducer (A392S, Panametrics, Waltham, MA, USA) with a

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diameter of 38 mm and a radius of curvature of 63.5 mm. The transverse focal width at half-maximum (FWHM) was about 3.0 mm, while the axial focal length was about 26 mm. The transducer was mounted on a removable cone filled with degassed water whose tip was sealed with a polyurethane membrane, and the center of the focal spot was at approximately 5 mm below the cone tip. The transducer was attached to a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA) to allow 3-D positioning. A function generator (33220A, Agilent Inc., Palo Alto, USA) was connected to a power amplifier (500-009, Advanced Surgical Systems, Tucson, AZ) to amplify the FUS excitation signal. The signal was sent through a custom-built electrical matching network (matched to a 50-Ω load) and, subsequently, to the FUS transducer. A power meter/sensor module (Bird 4421, Ohio, USA) was used to measure the input electrical power. The rat's head was mounted on the stereotaxic apparatus with the nose bar positioned 3.3 mm below the interaural line. Ultrasound contrast agent (UCA, SonoVue, Bracco International, Amsterdam, The Netherlands) was injected into the femoral vein of the rats before each sonication. Sonication was pulsed with a burst length of 50 ms at a 5% duty cycle and a repetition frequency of 1 Hz. Each sonication lasted 60 s. The ultrasound beam was delivered to the left brain hemisphere, centered on the tumor injection site. The sonication was at the acoustic power of 2.86 W with an injection of 300 µl/kg UCA.

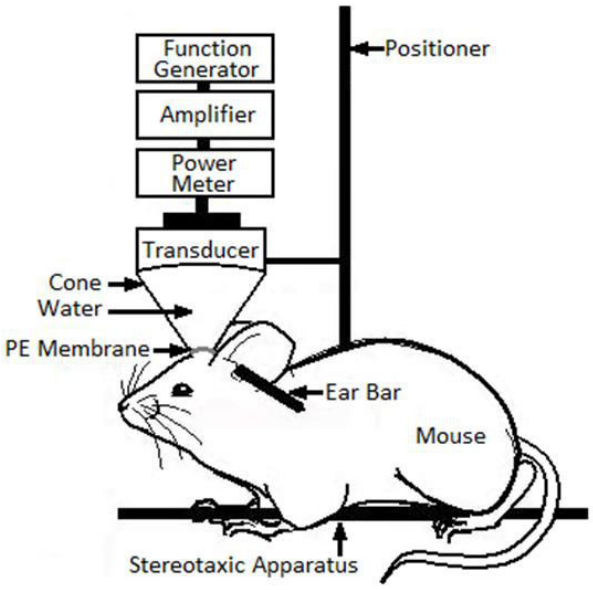

Fig. 1: Diagrams of the experimental setup for FUS-induced BBB disruption.

C. Lipoplatin Formulation

Preparation of lipid-encapsulated cisplatin was as following method. To mix cisplatin with DPPG at a 1:2 molar ratio in at least a 30% ethanol, 0.1 M Tri HCl, pH 7.5 to achieve about 5 mg/ml cisplatin concentration. And then, it is heating at 60ºC. The cisplatin-DPPG complex has improved properties over free cisplatin in tumor treatment. The cisplatin-DPPG complex is converted into liposomes encapsulating the cisplatin-DPPG by direct addition of premade liposomes followed by dialysis against saline and extrusion through menbranes to downsize these to 180-200 mm in diameter.

AP-1-conjugated DPPG-PEG was transferred into the preformed lipoplatin at a 1.5% molar ratio of total lipid components and incubated at 60ºC for 1 h to obtain AP-1-labeled lipoplatin (AP-1 Lipoplatin; Fig. 2). The resulting unconjugated lipoplatin and AP-1 lipoplatin were found to have particle diameters of 180 nm, as measured by a dynamic light-scattering apparatus (Coulter N4 plus, Beckman).

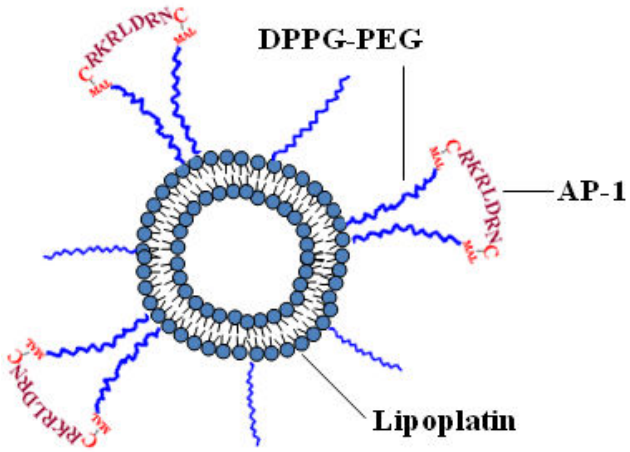

Fig. 2: Schematic diagram for the AP-1-conjugated liposomal cisplatin. Cisplatin molecules are surrounded by the lipid bilayer.

D. Treatment Procedure

The protocols of chemotherapy with or without FUS exposure were shown in Fig. 3. A control group of GBM-bearing mice received no treatment. The other GBM-bearing mice were divided into two groups and treated on days 6, 9, and 12 after tumor cell injection. One group was treated with lipoplatin alone. Another group received AP-1 lipoplatin followed by FUS sonication. The concentration of drugs that were administrated to the mice via tail vein injection was at the dose of 5 mg/kg.

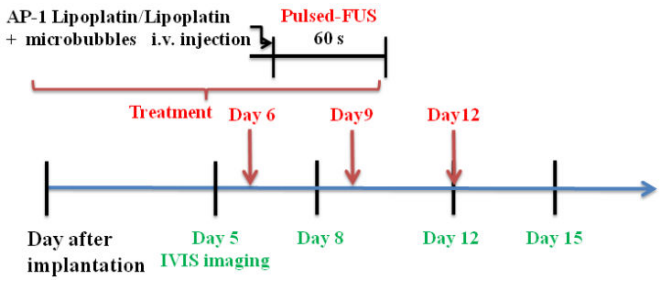

Fig. 3: The experimental time line for chemotherapy by lipoplatin alone or AP-1 lipoplatin with sonciation.

E. *Biophotonic Imaging*

Tumor size was quantified by biophotonic images obtained from 5 to 15 days after tumor implantation. The GBM8401 cell lines were transformed with the luciferase gene, and each mouse was injected with luciferin substrate. After anesthetic induction with isoflurane (1.5 l/min oxygen in 4% isoflurane), mice were imaged using the Xenogen IVIS imaging system (Xenogen, Palo Alto, CA, USA) with a 1-min acquisition time in small-bin mode. Luciferase activity was viewed and quantified using Living Image Software from Xenogen within a region of interest that encompassed the head of the mouse after administration of luciferin substrate to the anesthetized mouse.

III. RESULTS

The goal of this study was to investigate if the treatment of an established intracranial brain tumor derived from human GBM cells with targeted lipoplatin followed by pulsed FUS exposure could improve the efficacy of antitumor effect relative to administering the same dose of lipoplatin alone. In Fig. 4, GBM-bearing mice were treated with various protocols on days 6, 9, and 12 and tumor sizes were monitored by IVIS imaging from 5 to 15 days after tumor injection. Tumor cells grow rapidly in the untreated control group. Tumor growth on days 12 and 15 after implantation was suppressed in mice treated with lipoplatin alone. Tumor treatment with AP-1 lipoplatin followed by FUS sonication was more marked for inhibition of tumor cells growth.

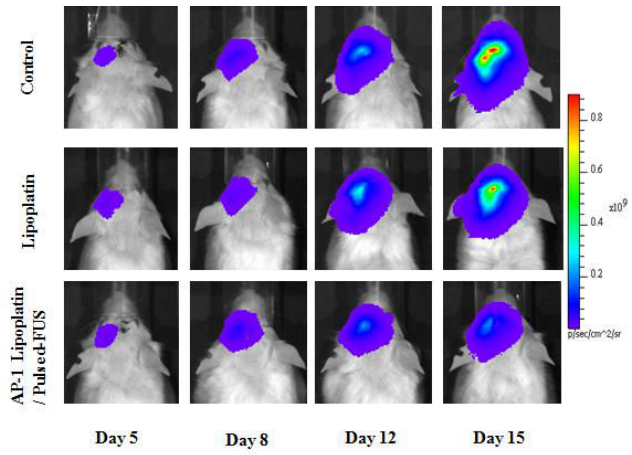

Fig. 4: Bioluminescence imaging of the GBM-bearing mice was detected for 5 to 15 days after tumor cells injection. Control tumor mice received no treatment. Second row showed the group of mice was treated with lipoplatin alone. Third row represent the mice received AP-1 lipoplatin followed by pulsed FUS exposure.

Figure 5 indicated that there was a decrease in the luciferase activity of tumor cells for the mice treated by lipoplatin alone or AP-1 lipoplatin in combination with FUS sonciation compared to the control untreated group. Furthermore, the tumor cells for the group of AP-1 lipoplatin with FUS were mildly lower than that of the mice treated with lipoplatin alone.

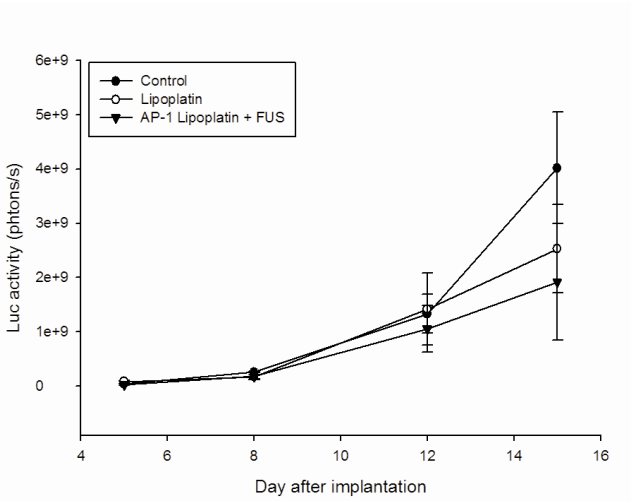

Fig. 5: Luciferase activity was viewed and quantified by analysis of bioluminescence images for various treatment procedures. (n=4 mice per group)

IV. DISCUSSION

The clinical application of cisplatin has been limited due to severe renal toxicity. Previous works reported that lipoplatin enhance the accumulation in the cancer cells but result in a lower cytotoxicity. The data suggest that carrying the cisplatin into liposome led to a different distribution of the drug in the cancer cells. Moreover, lipoplatin revealed the best cellular incorporation and reduced all the toxicities of cisplatin.

Our previous studies demonstrated that AP-1 liposomal doxorubicin with FUS exposure is able to achieve local high-dose chemotherapy for GBM and significantly improve the antitumor effect of the drug without increasing systemic toxicity. In this pilot study, the ligand-conjugated lipoplatin assisted by FUS has a modest improvement in the inhibition of tumor growth compared to the mice treated by lipoplatin alone. More investigations is needed to verify that the efficiency of chemotherapy enhanced by FUS sonication for various types of liposomal drugs in the future clinical applications.

Acknowledgment

This study was supported by grants from the National Science Council of Taiwan (no. NSC 101-2320-B-010-036-MY3), Cheng Hsin General Hospital Foundation (no. 101F195CY18 and 100F117CY25), Yen Tjing Ling Medical Foundation (no. CI-102-8 and CI-100-17).

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