Short Chain Elastin-Like Polypeptide-Incorporated Thermosensitive Liposome: In Vitro Evaluation and In Vivo Study Combined with High Intensity Focused Ultrasound (HIFU)

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ABSTRACT SUMMARY
A novel thermosensitive liposome with high stability was developed based on the incorporation of short chain elastin-like polypeptide (sELP) into thermosensitive liposome (STL). The doxorubicin (DOX) was encapsulated into the liposome by ammonium sulfate gradient loading method. The DOX release showed 95% at 42°C, mild hyperthermia condition, comparable with lysolipid-containing thermosensitive liposome (LTS). In vivo plasma stability of STL was considerably superior to LTSL. The anti-cancer efficiency of STL combined with HIFU exhibited significant tumor growth regression compared to others control group. These results imply that both of stability and thermosensitivity are important key parameter to improve the anti-cancer efficiency in mild hyperthermia therapy.

INTRODUCTION
The combination of regional mild hyperthermia and thermosensitive drug carrier offers a powerful tool for tumor specific drug delivery. High intensity focused ultrasound (HIFU) is able to heat at desired local site in clinical cancer treatment. The smart drug triggering system is realized by coupling HIFU and thermosensitive liposome reduces the damage to healthy tissue and improves therapeutic efficiency.

Lysolipid-containing thermosensitive liposome (LTS) has been developed by Needham’s group. LTS improved the drug release at the condition of mild hyperthermia (42–45°C) by inserting lysolipid such as MPPC (1-palmitoyl-sn-glycero-3-phosphocholine) into DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) bilayer [1]. Nevertheless, LTS exhibited drug leakage in physiological condition at 37 °C likely due to desorption of incorporated MPPC from lipid membrane [2]. The instability may induce drug release to healthy tissue and insufficient drug dose in tumor site.

To achieve both high stability and sufficient sensitivity to mild hyperthermia, we developed a novel thermosensitive liposome. To maintain stability, the packing lipid as membrane stabilizer was introduced to lipid bilayer. In addition, to increase temperature sensitivity, we incorporated elastin-like polypeptide (ELP) [VPGVG]n known as thermosensitive peptide. We selected three repeats of VPGVP resulting from the screening of relevant short chain ELP (data not shown). The [VPGVG]3-lipid conjugate was incorporated into liposome bilayer (Fig. 1A). We tested drug release profile of the short chain ELP-incorporated thermosensitive liposome (STL) and demonstrated the promising in vivo results of STL combined with HIFU.

EXPERIMENTAL METHODS
Liposome was prepared by the lipid film hydration and extrusion method [3]. Encapsulation of DOX into STL was carried out using ammonium sulfate gradient-driven loading method. Liposome consists of 1,2-dipalmitoyl-sn-glycerol-3-phosphocholine (DPPC), 1,2-distearyl-sn-glycerol-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DSPE-PEG), cholesterol, and modified ELP with mole ratio of 55:2:10:0.55. LTSL was prepared as a control procedure of reference [4].

Drug release pattern was measured using the fluorescent quenching property of DOX. Each aliquot of liposome suspension was incubated in a preheated chamber for 5 min, and the fluorescent intensity was monitored at 615 nm (Ex. 490 nm) by a fluorescence spectrometer (PerkinElmer, Envision 2104-multilabel reader). The drug release was calculated as the relative percentage of 100% control (liposome in 1% TritonX100 in ethanol.)

To determine the plasma circulation time, DOX-loaded STL and LTSL (5 mg of DOX/kg) were injected into BALB/c mice via the tail vein. Blood was collected from the tail vein at various times (30 min to 24 hr). Plasma was isolated from the blood by centrifugation (4 °C, 15 min 2500 rpm) and diluted (1/100) with acidified isopropyl alcohol. The mixture was incubated at 4 °C overnight. The sample was centrifuged (10 min, 12,000g) and fluorescence of the supernatant was determined at Ex 490 nm/Em 615 nm.

In vivo anti-cancer efficiency experiments were performed on the 8th day after tumor cell injection, when the average tumor volume was approximately 250 mm³ (7-8 mm in diameter, ±25%). Immediately before HIFU
treatment (Therapy and Imaging Probe System, Philips Research, Fig. 1B), drugs (5 mg/kg of free DOX) or its equivalent were administered via tail vein to mice. 4 spots (2x2, raster grid pattern) of HIFU focus were used for sonication with an interval of 3 mm between each spot to cover the tumors maintaining for 15 min per spot (i.e., 60 min for a mouse).

RESULTS AND DISCUSSION

To evaluate the thermal sensitivity of DOX-loaded STL (STL-DOX) and DOX-loaded LTSL (LTSL-DOX), drug release profile was tested (Fig. 2). The amount of released DOX after 5 min incubation in 20% serum-containing cell media was measured in the temperature range 25–50 °C. STL-DOX gave rapid drug release from 39 °C, with a maximum (≥ 95% release of DOX) at 42 °C. The DOX release profile of STL and LTSL were similar, but the response of STL was more temperature-sensitive than LTSL.

![Figure 2. Release profile of DOX from STL and LTSL in 20% serum containing culture media.](image)

The pharmacokinetics of STL-DOX was monitored. After intravenous injection of STL-DOX, the blood was collected at each time point, and the plasma level of DOX was determined (Fig. 3). After 2 hr of LTSL-DOX injection, DOX level in the blood rapidly fell, and < 20% of the injected dose (ID) was observed after 5 hr (t1/2 = 2.7 hr). In contrast, STL-DOX was maintained at 80% of ID after 5 hr, and DOX plasma level fell gradually (t1/2 = 8 hr). The biodistribution study also showed no significant difference in DOX concentration in muscle, heart, liver, spleen, and kidney in any group (free DOX, LTSL-DOX, and STL-DOX) (data not shown). However, the amount of STL-DOX in blood was much higher than that of free DOX and LTSL-DOX, by a factor of 3.5. The biodistribution data are consistent with the pharmacokinetics profile, which showed high stability of STL in the blood.

![Figure 3. Blood circulation time of DOX in DOX-loaded STL and LTSL in mice.](image)

We evaluated the capability of STL-DOX to inhibit tumor growth. STL-DOX with HIFU suppressed tumor growth in most of the injected mice after a single-dose treatment. In particular, the mice treated with STL-DOX/HIFU alone showed tumor regression for initial three days among the groups tested. LTSL-DOX/HIFU also inhibited tumor growth to a moderate extent. In all groups, HIFU-treated tumors fared better than untreated ones. STL-DOX/HIFU group displayed better anticancer efficacy than the others because the STL led to faster thermosensitive drug release capability at 39 - 42 °C, and also because of the highly stable property, sufficient to retain drug molecules inside the liposome. As a result, STL has a greater chance of being delivered to the tumor over time. LTSL-DOX exhibited similar anticancer efficacy regardless of HIFU, because the low stability of LTSL formulation in blood is liable to induce drug leakage before reaching the tumor site.

![Figure 4. Anticancer efficacy of DOX-loaded STL or LTSL. *, p<0.05, significant difference compared with C.](image)

CONCLUSION

We have developed a highly thermo-responsive liposome with high in vivo stability by fabricating DPPC, DSPE-PEG2000, cholesterol, and ELP. STL formulation was stable at physiological temperatures but gave a significant release of encapsulated drug after a short period of mild heating. The in vivo pharmacokinetics result proved the stability of STL. Antitumor efficacy study of STL in combination with HIFU gave therapeutically promising results. Further optimization is now looking at the effects of thermal doses, of lag time between intravenous administration and hyperthermic therapy, and of treatment duration on antitumor efficacy.

REFERENCES