Thermosensitive Liposomes for the Localized Delivery of Gemcitabine and Oxaliplatin

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ABSTRACT SUMMARY

Thermosensitive liposomes (TSLs) provide a triggered delivery system for the localized delivery of chemotherapeutic drugs to tumors. This technology is showing potential in late stage clinical trials for recurrent breast cancer and hepatocellular carcinoma (ThermoDox®, Celsion Inc.), and if successful this could lead to an increased demand for these types of treatments in the clinic.

TSLs have generally been applied to membrane permeable drugs, such as doxorubicin (DOX), and it seems this concept has not yet been studied extensively for formulations of other less membrane permeable drugs.

HaT is a novel TSL formulation developed by us, and here we describe our findings using it for the delivery of gemcitabine (GEM) and oxaliplatin (OXA). Pharmacokinetic (PK), biodistribution and efficacy data has been studied and the key findings are discussed.

INTRODUCTION

The delivery of a drug to its target with minimal systemic toxicity is a major goal of drug delivery research. Through the use of TSLs in combination with advanced image-guided heating methods, the potential of specific and localized delivery is becoming more of a reality.1

TSLs are designed to release their payload at mild-hyperthermic temperatures (39-43°C).2 For optimal performance this heating is applied to just the tumor target, leaving the rest of the vasculature at normal physiological temperature. In this way, the TSL remains stable at 37°C as it passes through the systemic circulation and the drug is protected by the liposomal membrane allowing increased drug to flow through the bloodstream by minimizing clearance and non-specific uptake. When the TSL reaches the heated tumor (39-43°C), it releases its contents rapidly, causing a high local concentration of drug within the vasculature of the tumor. At these high concentrations the drug penetrates into the tumor and can act via understood mechanisms to bring about a therapeutic response.

This concept of TSL mediated drug delivery has been studied previously and is now being investigated in clinical trials for a number of different cancer indications. The trailblazing formulation of this type is LTSL (Lyosolpid Temperature Sensitive Liposome, DPPC/MSPC/DSPE-PEG2000, 86/10/4 mol%) also known as ThermoDox, which will reach the end of a phase III trial for hepatoacellular carcinoma later this year (www.clinicaltrials.gov, ID: NCT00617981; other clinical trials for this formulation are also ongoing).3,4

To date, LTSL and other therapeutic TSL formulations have mainly been studied for the delivery of DOX, a highly membrane permeable cytotoxic. However, DOX is a known cardio toxin, and lifetime doses of DOX are therefore limited.

For this reason we are interested in developing TSLs suitable for the delivery of other drugs which will not compound the problem of potential DOX cardiotoxicity and will also be suitable for DOX-resistant indications. In particular, we have chosen GEM and OXA as drugs to investigate for delivery via a TSL mediated strategy. Rather than use LTSL, we have developed a simpler 2 component TSL called HaT (Heat activated cytoToxic, DPPC/Brij78, 96:4 mol%). Originally this formulation was developed to deliver DOX, which showed faster drug release at mild-hyperthermic temperatures and improved drug stability at 37-38°C compared to LTSL.5,6,7,8 These improvements led to further enhanced tumoral drug uptake and efficacy in vivo relative to LTSL. HaT has been used here to deliver GEM or OXA via a burst release action in heated tumors.

EXPERIMENTAL METHODS

LTSL was formulated with 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1-stearoyl-2-hydroxy-sn-glycero-3-phosphatidylcholine (MSPC) and 1,2-distearyloyl-sn-glycero-3-phosphatidylethanol-amine-N-[methoxy (polyethyleneglycol)-2000] (DSPE-PEG2000) in a 86/10/4 molar ratio. HaT was formulated with DPPC and Brij78 (polyethoxyethyene stearyl ether) in a 96/4 molar ratio. All liposomes were prepared by thin film hydration with a ~10 mg/mL solution of drug (GEM or OXA) in buffer. Each formulation was extruded to size, and the outer phase was exchanged with buffer by dialysis. The drug concentration was determined after the disruption of liposomes with Triton X-100. Drug concentrations were analyzed by HPLC-MS (GEM) and ICP-AES (OXA) methods. Encapsulation efficiency of all passively loaded drugs was ~10%.

Drug release models: Liposomes (~200 µg/mL Drug) in release buffer were incubated at different temperatures (37-42°C) for given times, then immediately put on ice. Samples were diluted with buffer, filtered, and analyzed for released drug (HPLC-MS or ICP-AES).

In vivo studies: female BALB/c mice (aged 5-6 weeks, 18-20 g) were purchased from The Jackson Laboratory (Bar Harbor, ME). All experimental protocols in this study were approved by the Animal Care Committee of the University Health Network (Toronto, Ontario, Canada) in accordance with the policies established in the Guide to the Care and Use of
Experimental Animals prepared by the Canadian Council of Animal Care. Briefly, PAN02 or EMT-6 cells were s.c. implanted into a leg of C57BL6 or BALB/c mice respectively, and 7 days post-tumor inoculation (tumor mass was approximately 0.2-0.3 g), the mice were anesthetized, and the tumor bearing leg was immersed in a water bath maintained at 43°C. The tumor was heated for 10 min for temperature equilibration before the i.v. injection of different drug formulations, and then heating for a further hour. For the biodistribution study, blood, heart, kidney, liver, lung, spleen and tumors were harvested following the treatment. For studies with GEM the tissue was homogenized and an aliquot of the supernatant was studied by HPLC-MS. For OXA experiments, the samples were digested with concentrated acid and then studied for Pt concentration by ICP-AES.

For the efficacy study, PAN02 or EMT-6 tumor-bearing mice were treated as above, and tumor size and body weight were monitored until endpoint.

RESULTS AND DISCUSSION

HaT-GEM and LTSL-GEM formulations were prepared and showed similar physical characteristics (size ~100 nm, PDI < 0.1).

We encountered problems preparing formulations of LTSL with OXA solutions using the passive hydration method. Investigations into the cause of this are ongoing, but at present we believe it may be related to the incompatibility of the LTSL lipid composition and the low NaCl concentration necessary for working with OXA (5% dextrose was the buffer used). For this reason, no comparisons between HaT-OXA and its LTSL counterpart could be made. HaT formulations of OXA were formed in the usual way.

HaT-GEM released >80% GEM within 1 min at 41-42°C, with <5% drug leakage at 37°C after 30 min in serum, while LTSL-GEM was stable at 37°C but exhibited slower release at 41°C (1%) and 42°C (~70%). This can be represented as 1.5 to 8-fold decreased release rate constants at 41-42°C for LTSL relative to HaT under the same conditions.

The PK profile of GEM was improved for both liposomal formulations with ~100% injected dose (ID) left in the blood after 1 h and ~9% remaining after 4 h. This was compared to ~1% and 0.03% ID respectively for free GEM. HaT-GEM improved drug delivery to the heated tumor relative to LTSL-GEM by 7.5-fold, and significantly enhanced antitumor efficacy with complete inhibition of tumor growth after a single dose of HaT-GEM (Figure 1a).

The release rates of HaT-OXA were rapid at 41-42°C, with >80% released within the first minute, but <5% release at 37°C. HaT-OXA displayed an enhanced retention of drug in the blood with ~15% ID after 1 h compared to ~2% for free OXA. Antitumor efficacy was considerably improved relative to free drug, with minimal tumor growth after 7 days following a single dose (Figure 1b).

CONCLUSION

Here we describe the successful formulation of HaT-GEM and HaT-OXA TSLs. Where possible we have compared the HaT formulations with LTSL formulations of the same drug. The HaT formulations display improved PK, increased drug uptake and improved efficacy relative to the free drugs.

These formulations may provide a useful treatment for situations where DOX is either ineffective or where patients have reached maximum exposure levels of DOX already.

REFERENCES

2. May, J.P.; Li, S.D.; Recent Patents on Biomedical Engineering 2012, 5, 148-158

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