Optimization of thermosensitive liposome formulations for intravascular drug release

W.J.M. Lokerse¹, T.L.M. ten Hagen¹, and G.A. Koning¹,²

¹Innovative Targeting Group, ²Laboratory Experimental Surgical Oncology, Department of Surgery, Erasmus MC Cancer Institute, Rotterdam, PO Box 2040, 3000CA The Netherlands.

Abstract Summary
Thermosensitive liposomes (TSL) for intravascular doxorubicin (Dox) release should be stable at 37°C and release Dox at an optimal rate at 42°C. Optimization involved studying the effect of drug loading gradients and various TSL lipid compositions on loading efficiency, particle characteristics, stability and triggered release in serum. Furthermore, the intravascular release pattern of these formulations was examined in murine dorsal skinfold window studies.

Introduction
Cancer chemotherapy is limited in efficacy and causes major side-effects. As a result, research aims at improving tumor drug delivery. The use of small liposomes (80 nm) loaded with high concentrations of chemotherapeutic drugs, provides important advantages over free drug administration. These carriers have already been introduced into the clinic as “Doxil”, a long circulating liposome formulation loaded with Dox. Although this formulation greatly decreased Dox toxicity, controlled drug release at the tumor site remains a major obstacle. The aim of our work is to design liposomes with thermosensitive bilayers and use mild hyperthermia at 42°C to trigger drug release in tumors. Intravascular drug release from circulating TSL is an attractive novel approach for efficient drug delivery [1,2].

For this approach TSL are hypothesized to require ultrafast release kinetics upon hyperthermia, which normally coincides with significant premature drug release at physiological temperature [2]. In this study, we varied TSL lipid composition and drug loading strategies in order to balance these two seemingly conflicting TSL requirements. For these TSL we investigated drug-loading, particle characteristics (size homogeneity and morphology), stability and temperature- and time dependent release kinetics. In vivo Dox delivery from TSL in tumor vasculature was examined using a murine dorsal skinfold tumor model and intravital microscopy.

Experimental Methods
Liposomes were prepared using the film hydration and extrusion method. In short, lipids were dissolved in chloroform/methanol and thin lipid film was produced using a rotary evaporator. The film was hydrated in either ammoniumsulfate (pH 5.5) or citrate (pH 4)
and extruded five times through polycarbonate filters of 200, 100, 80 and 50 nm pore size. These liposomes were analyzed for size and zeta potential by dynamic laser light scattering (Zetasizer, Malvern Instruments) and morphology using cryo-TEM. Dox was loaded using a citrate- or ammoniumsulfate-based pH gradient [4]. Temperature dependent release was analyzed by 5 min exposure of TSL to varying temperatures in FBS. Time dependent release analysis was performed by exposing the liposomes to 37°C or 42°C for 1 hour in FBS. In vitro examination of intravascular release was monitored by intravital fluorescence microscopy on dorsal skin flap window chamber models in mice implanted with B16 murine melanoma.

Results

By varying lipid composition, we have produced 4 TSL formulations that displayed 50% Dox release levels (T_{50}) with approximately 1 °C increase of temperature (Table 1). All TSL formulations showed 100% Dox loading efficiency using ammoniumsulfate or citrate pH gradient loading [Methods 3,4]. All TSL formulations remained stable in size and showed no leakage during 2 months of storage. In time and temperature dependent release experiments, ammoniumsulfate loaded TSL demonstrated faster release at 42 °C and higher stability at 37°C. By fine-tuning TSL lipid composition we obtained a formulation which shows complete and rapid Dox release when exposed to mild hyperthermia and no release at 37°C.

Preliminary intravital imaging studies in mice bearing dorsal skinfold window chambers with implanted B16 tumors confirmed temperature-induced release patterns that were found in vitro, in an actual tumor environment.

<table>
<thead>
<tr>
<th></th>
<th>T_{50} release (% in 5 min)</th>
<th>Release 1h 37°C</th>
<th>Release 1h 42°C</th>
<th>Size (Pdi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ufTSL</td>
<td>39.2°C</td>
<td>15%</td>
<td>100%</td>
<td>81nm (0.056)</td>
</tr>
<tr>
<td>fTSL</td>
<td>40.5°C</td>
<td>0%</td>
<td>100%</td>
<td>82nm (0.057)</td>
</tr>
<tr>
<td>imTSL</td>
<td>41.5°C</td>
<td>0%</td>
<td>94%</td>
<td>82nm (0.039)</td>
</tr>
<tr>
<td>sTSL</td>
<td>42.7°C</td>
<td>0%</td>
<td>72%</td>
<td>81nm (0.058)</td>
</tr>
</tbody>
</table>

Table 1: Characteristics of ultrafast TSL (ufTSL), fast TSL (fTSL), intermediate TSL (imTSL) and slow TSL (sTSL). The second column shows the temperature where 50% of Dox is released after 5 min in serum. The third and fourth columns show the percentage release after a 1 h exposure to 37°C or 42°C, respectively. The last column gives the size of the liposomes and the polydispersity index (Pdi).

Conclusion

We have succeeded in obtaining a TSL formulation which shows no drug release at physiological temperatures and instant and complete release when exposed to mild hyperthermia. Future studies will investigate efficacy of this formulation in various mouse tumor models.

References:

2: Li et al. J. Control Rel. 2013
4: Mayer et al. Biochim Biophys Acta. 1986