Liposomes for triggered drug delivery: effect of lipid composition on the sensitivity of liposomes to 1.1 MHz ultrasound
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ABSTRACT SUMMARY
High frequency (1.1 MHz) ultrasound (US), may be used as an external trigger for targeted drug delivery to tumors and relevant organs. One approach is to trigger release of drug from US-sensitive liposomes but the US itself should not cause damage to healthy tissue. Consequently, the challenge is to create highly US-sensitive liposomes and this requires an understanding of the mechanisms by which US disrupts liposomes. This paper reports the effect of lipid composition on US-sensitivity of liposomes and explores mechanisms (heating, membrane compressibility) for the sensitivity.

INTRODUCTION
US has been shown to trigger drug release from liposomes (1). Liposomes differ in their US-sensitivity but the relationship between the physicochemical properties of liposomes and their US-sensitivity is not clear. Such an understanding is fundamental for the development of this triggered drug delivery strategy.

Some physicochemical properties that have been postulated include the incorporation of either thermally-sensitive constituents (eg DPPC) or membrane destabilisers (eg DOPE). These modifications rely on the ability of US to cause heating and/or mechanical perturbations which either lead to rupture of liposomes or increased permeability of the liposomal wall (2).

This paper explores these mechanisms and cautiously concludes that thermally-sensitive liposomes are a better option for further studies.

EXPERIMENTAL METHODS
DSPC-Cholesterol (Chol) (87-13 molar ratio) liposomes containing carboxyfluorescein (CF, 50 mM), were prepared by the thin film method, extruded (200 nm polycarbonate membrane), and separated from free CF by gel (Sephadex G50) chromatography. US-sensitive liposomes DOPE-DSPC-Chol (30-57-13) and DPPC-DSPC-Chol (67-20-13) were prepared similarly.

Liposomes (2 mL) were exposed to 1.1MHz (1.5 W/cm\textsuperscript{2}) US for 1, 2, 4, 6, or 10 min at 37\textdegree C. After exposure, the amount of CF released was quantified by a validated spectrofluorimetric assay and compared with controls not exposed to US. Release of CF from liposomes not exposed to US was studied at 37 and 42\textdegree C

To assess the flexibility of the lipid membranes, Langmuir trough studies were conducted using a Langmuir-Blodgett trough (100 cm\textsuperscript{2}) with a Wilhelmy paper plate at ambient temperature. MilliQ water (50 mL) was used as the subphase. The lipid monolayers included the liposome lipid combinations and DOPE-Chol (87-13). A 10-20\textmu l aliquot of 1 mM lipid mixture in chloroform was spread on the subphase and after allowing 10 min for solvent evaporation, compression was started at a speed of 10 cm/min and the surface pressure ($\pi$) - area (A) isotherm recorded. Surface compressional modulus (K), which is inversely related to flexibility, was calculated as:

$$K = -\frac{A}{\pi} \frac{\partial^2 \pi}{\partial A^2}$$

where $A$ is the area per molecule at surface pressure $\pi$.

RESULTS AND DISCUSSION
The effect of phospholipid composition on the mechanical properties of the bilayers, was modeled using Langmuir trough monolayers. Phospholipid composition had a clear effect on the compressional behavior of the monolayers (Figure 1). These data were converted using cubic splines and Equation 1 into compressional
moduli which indicate that DOPE lipids have the lowest compressional modulus (ie highest flexibility) (Figure 2).

Figure 1: Pressure area isotherms of phospholipid compositions

Figure 2: Compressibility profiles of relevant monolayers

Liposomes of different lipid compositions were tested for their US-sensitivity. The DPPC formulation was the most sensitive to 1.1MHz US whereas incorporation of DOPE did not increase (p>0.05) the sensitivity of DSPC-Chol liposomes (Figure 3) inspite of its effect on bilayer flexibility. DOPE has been shown to increase the sensitivity of liposomes to 40 kHz US (3).

Since US increased the temperature of the liposome suspension in the sample chamber by 2.5 °C (±0.5) in less than 2 minutes, the effect of temperature without US was studied (Figure 4) DPPC liposomes were most sensitive to temperature increase. A comparison of data in Figures 3 & 4 indicates that US is heating liposomal membranes leading to a phase change in the DPPC (Tm=41°C) and subsequent increase in permeability of the bilayer.

Figure 3: Effect of US and bilayer composition on release of CF from liposomes. Y axis shows the increase in release caused by US compared with release from liposomes not exposed to US (Data are means ± SD, n=6)

Figure 4: Effect of temperature on the release of CF from liposomes. Y-axis is the difference in release at 37 °C and 42 °C (Data are means ± SD, n=6)

CONCLUSION

Liposomes containing DPPC are triggered to release drug with 1.1MHz US whereas those containing DOPE are less sensitive. The release is due to heating to temperatures which are physiologically acceptable using US which itself does not damage tissue. Temperature-sensitive liposomes are a better option for further studies.

REFERENCES

