Polymeric Micellar Carriers for a Hydrophobic Anti-cancer Drug

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ABSTRACT SUMMARY
Polymeric micelles (PMs) were formulated to solubilise the hydrophobic anti-cancer drug, SN25860. By increasing the polymer concentration, the amount of drug loaded in the formulation was increased. The drug-loaded PMs were 26-28 nm in size with narrow size distributions. The entrapment efficiency of the formulations was above 90%. A sustained release profile and minimal in-vitro haemolysis demonstrated suitability for parenteral administration. The PMs may be considered a suitable carrier for the anti-cancer drug SN25860.

INTRODUCTION
Cancer is a leading cause of morbidity and mortality globally.1 Despite decades of research, conventional chemotherapies have achieved limited clinical success as anti-cancer drugs are often characterized by poor specificity, resulting in high systemic toxicity, with poor aqueous solubility making them difficult to formulate.2

In order to improve the performance of anti-cancer drugs, targeted drug delivery systems are being developed. Among these, PMs have three critical functions; drug solubilisation, drug targeting, and controlled release of drug.3, 4 PMs also have the attractive properties of simple preparation, good stability, deeper tumour penetration and higher drug loading capacity than other carrier systems. They are formed by spontaneous self-assembly of amphiphilic block copolymers. Typically, PMs are spherical in shape, sized from 10 to 100 nm, with a hydrophobic core-hydrophilic shell structure.3, 4

In this study, PM formulations based on Pluronic copolymers are developed to enhance the solubility of a hydrophobic dinitrobenzamide mustard hypoxia-activated prodrug SN25860 (intrinsic solubility of the sodium salt 50 μg/mL) developed at the University of Auckland. The formulations were characterised for suitability for parenteral delivery, and safety was assessed using a rapid in vitro haemolysis test.

EXPERIMENTAL METHODS
Drug-loaded PM (using the sodium salt of the drug) was formulated by the thin film hydration method. A mixture of Pluronic® P123 (Sigma-Aldrich) and Pluronic® F127 (BASF) at a volume ratio of 2:1 was used to prepare drug loaded PM with varying polymer concentrations. The polymers and drug were dissolved in acetonitrile:methanol:chloroform (0.75:2.25:1). The solution was evaporated in a rotary evaporator at 50°C and the resultant thin film hydrated with 5% glucose solution (v/v) at 50°C for 30 mins. The final product was then stored at 4°C.

Particle size and zeta potential were determined by Zetasizer. The morphology was observed by transmission electron microscope (TEM) following dilution with water and negative staining with 2% uranyl acetate. Drug concentration was determined by high performance liquid chromatography (HPLC) utilizing a reversed phase C18 column set at 25°C. Acetonitrile and 50 mM (pH 3) Na2HPO4 buffer (45:55, v/v) was used as mobile phase at a flow rate of 1 ml/min. The UV detection wavelength was 360 nm. Drug loading (DL) and entrapment efficiency (EE) were calculated as follows:

\[
DL(\%) = \frac{\text{Mass of the drug in micelles}}{\text{Total mass of the polymers and drug}} \times 100
\]

\[
EE(\%) = \frac{\text{Mass of the drug in micelles}}{\text{Total mass of the drug used in micelle preparation}} \times 100
\]

Thermal analysis of the formulations was carried out by differential scanning calorimetric (DSC) testing from -20°C to 250°C at 10°C/min. For DSC testing the formulations were freeze-dried.

In vitro drug release was studied by placing 3 ml of drug-loaded PM in dialysis tubing and submerged in 200 ml of 0.1 M PBS, pH 7.4 at 37°C. At specific time intervals, 1 ml of sample was withdrawn and replaced with an equal volume of fresh medium.

An in vitro haemolysis test was used to evaluate biocompatibility for parenteral use. The formulations were incubated with equal volumes of 4% (v/v) rat red blood cell (RBC) suspension at 37°C for 30 minutes. The percentage of haemolysis was determined by UV absorbance measurement using a 96 well plate reader.

RESULTS AND DISCUSSION

1. Particle size, PDI and zeta potential
Increase in the polymer concentration was associated with decreased micellar size for blank formulations, but had little effect on zeta potential (Table 1). The zeta potential decreased when drug was loaded in the formulations, while the polydispersity index (PDI) remained relatively unchanged. Interestingly, for the lowest polymer concentration (48 mg/mL), PM size decreased (p<0.05) when drug was loaded but at higher polymer concentrations PM size increased slightly when drug was loaded. The decrease in size at low polymer concentrations may be due to the polymer concentration being insufficient to form complete micelles. On incorporation of the hydrophobic drug, the polymers may have formed micelles more willingly, thus the size of PMs became smaller. The slight increase in size at higher polymer concentrations being insufficient to form complete micelles.

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concentrations may be due to the increase in micelle core size caused by incorporation of the drug.

Table 1: Diameter, PDI and zeta potential of drug-loaded and blank PM (mean± SD, n=3)

<table>
<thead>
<tr>
<th>Total polymer concentration (mg/ml)</th>
<th>Diameter (nm)</th>
<th>PDI ± SD</th>
<th>Zeta potential (-mV) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>48 Blank PM</td>
<td>46.82 ± 4.38</td>
<td>0.178 ± 0.013</td>
<td>15.6 ± 7.23</td>
</tr>
<tr>
<td>F (Ori)</td>
<td>27.34 ± 1.12</td>
<td>0.201 ± 0.015</td>
<td>11.7 ± 3.16</td>
</tr>
<tr>
<td>96 Blank PM</td>
<td>24.44 ± 1.82</td>
<td>0.138 ± 0.046</td>
<td>10.8 ± 3.44</td>
</tr>
<tr>
<td>F2</td>
<td>26.18 ± 2.48</td>
<td>0.137 ± 0.044</td>
<td>4.12 ± 2.20</td>
</tr>
<tr>
<td>144 Blank PM</td>
<td>23.57 ± 0.83</td>
<td>0.119 ± 0.018</td>
<td>11.0 ± 0.21</td>
</tr>
<tr>
<td>F3</td>
<td>28.02 ± 2.70</td>
<td>0.162 ± 0.023</td>
<td>9.55 ± 0.98</td>
</tr>
</tbody>
</table>

2. Morphology

Both blank and drug-loaded formulations were spherical in shape with diameters consistent between TEM observation (Fig. 1) and zetasizer measurement. Flocculation of particles in the blank PM formulation was revealed by TEM.

3. Entrapment efficiency and drug loading

The EE and DL of all formulations were 90% and 2% respectively. The drug concentration increased in a linear manner to the polymer concentration (Fig. 2). The results showed that the PMs were able to improve the solubility of SN25860 to 3 mg/ml, which is significant for a hydrophobic drug. The linearity and the negative intercept in Fig 2 may indicate the drug has been fully incorporated in the PM with the ability to improve DL by adding more drugs.

CONCLUSIONS

The PM formulations enhanced the aqueous solubility of the hydrophobic drug SN25860. This formulation has a small size (~27 nm), and is suitable for tumour penetration. The formulation demonstrated sustained drug release, and minimal haemolysis, thus is a promising formulation for parenteral administration in preclinical trials.

REFERENCES

1. C Gong et al., Biomaterials 2013, 34, 1413.

ACKNOWLEDGMENTS

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