Methacrylamide-oligolactates as building blocks for targeted biodegradable polymeric micelles to deliver photosensitizers

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Introduction

Amphiphilic block copolymers consisting of poly(ethyleneglycol) (PEG) and p(methacrylamide-oligolactates) self-assemble in aqueous media into spherical micelles above the cloud point (CP) of the thermosensitive block [1,2]. Cleavage of the lactic acid side chains causes a gradual increase of the critical micelle temperature (CMT) and leads to controlled destabilization of the micelles (Figure 1).

Figure 1 General concept of thermosensitive biodegradable polymeric micelles.

The hydrophobic micellar core and its destabilization at physiological conditions can be exploited for targeted delivery of hydrophobic drugs at their site of action. The hydrophobicity of many photosensitizers, which are applied in photodynamic therapy (PDT), results in severe side effects and necessitate that they are encapsulated in an adequate carrier system to deliver them specifically to tumour regions (Figure 2).

Figure 2 Photodynamic therapy.

Aim

The application of biodegradable thermosensitive polymeric micelles and their use for targeted delivery of photosensitizers to cancer cells.

Synthesis monomers and polymers

Monodisperse methacrylamidoalkyl oligolactates were synthesized in a stepwise manner [1] (Scheme 1).

Scheme 1 Step by step synthesis of monodisperse methacrylamido-oligolactates. R₁ and R₂ are H or CH₃. PEG = protecting group

Free radical polymerization of these monomers with either the macroinitiator (CH₂=CH-EG)₉ or ABCPA or (NH₃-PEG₃)-ABCRA (ABCRA = 4,4’-azobis(4-ynamino-n-propanoic acid) resulted in amphiphilic thermosensitive block copolymers.

Figure 3 Chemical structure of a R₃-P(MEG₃-b-p(HPMAm-Lac₃)) block copolymer. n is 1 to 4, R₁ = OCH₃ or NH₃, R₃ = H or CH₃.

Targeted Micelles

The primary amines on the micellar surface can be used to covalently link, via a bifunctional coupling agent, targeting ligands (e.g. antibodies).

Encapsulation silicon phthalocyanine

A newly derivatized silicon phthalocyanine (SiPc) with maximum absorbance at 674 nm and high photocytotoxicity (IC₅₀ = 0.1 μM [4]) was entrapped in mPEG₃-b-p(HPMAm-Lac₄) micelles (Table 2).

Table 1: Characteristics of some amphiphilic block copolymers and their micelles based on mPEG₃-b-p(HPMAm-Lac₄).

<table>
<thead>
<tr>
<th>Polymer composition</th>
<th>Mₚ (g/mol)</th>
<th>CMT (°C)</th>
<th>CMC* (mg/ml)</th>
<th>Zₐ (nm)</th>
<th>Destabilization time period (in hours; pH 7.4, 37°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mPEG₃-b-p(HPMAm-Lac₄)</td>
<td>19000</td>
<td>10</td>
<td>0.015</td>
<td>60</td>
<td>166</td>
</tr>
<tr>
<td>mPEG₃-b-p((20%HEMAm-Lac₄)-(80%HEMAm-Lac₃))</td>
<td>24000</td>
<td>6</td>
<td>0.08</td>
<td>80</td>
<td>8</td>
</tr>
</tbody>
</table>

* CMC = critical micelle concentration

Whereas the solubility of the free phthalocyanine in water is negligible, these micelles were capable of solubilizing 0.15 mg phthalocyanine per mL.

Table 2: Effect of incorporation of a silicon phthalocyanine on the particle size of mPEG₃-b-p(HPMAm-Lac₄) micelles and the encapsulation efficiency.

<table>
<thead>
<tr>
<th>SiPc (mg/mL)</th>
<th>Zₐ (nm)</th>
<th>PD %</th>
<th>SiPc encapsulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>64</td>
<td>0.01</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>78</td>
<td>0.07</td>
<td>-</td>
</tr>
<tr>
<td>0.5</td>
<td>74</td>
<td>0.24</td>
<td>84</td>
</tr>
<tr>
<td>1.5</td>
<td>*</td>
<td>*</td>
<td>69</td>
</tr>
<tr>
<td>2.5</td>
<td>*</td>
<td></td>
<td>61</td>
</tr>
</tbody>
</table>

* Could not be measured due to absorption of the DLS laser light by the SiPc.

In vitro studies show that at a low pH where the micelles are stable, the photosensitizer remained in the micellar core (Fig 4, left). On the other hand at pH 8.5, where rapid hydrolysis of the lactic acid side chains occurs, the micelles destabilize which is associated with release of the phthalocyanine (Fig 4, right).

Conclusion

- R1-P(PEG₃-b-p(HPMAm-Lac₄)) are thermosensitive amphiphilic block copolymers which form micelles (60-80 nm) above the cloud point.
- This carrier system was capable of encapsulating a hydrophobic silicon phthalocyanine up to a final concentration of 0.15 mg/mL.
- The phthalocyanine was only released once the micelles were destabilized.
- Further research involves biodistribution studies of the photosensitizers in the (targeted) micelles after i.v. administration and the therapeutic efficacy of the photosensitizer-loaded targeted micelles.

References

4. Hofman, J.W., et al., to be published.

Acknowledgements

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