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Biodegradable microspheres for the sustained release of PDGFβ-receptor directed pPB-HSA targeted to the fibrotic kidney

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Introduction

Fibrosis

Fibroblast Derived Growth Factor (PDGF) plays a key role in the development of fibrotic processes in several tissues. Accordingly, the PDGFβ-receptor is abundantly present in these fibrotic tissues.

The cyclic peptide pPB

- pPB is a cyclic receptor binding peptide that contains the binding site of endogenous PDGF-BB.
- Coupling the cyclic peptide pPB to human serum albumin (HSA) prevents rapid renal excretion and allows for a better receptor presentation.
- The cyclic peptide can bind to the PDGFβ-receptor without eliciting a response.
- pPB-HSA can be used as a carrier to target therapeutic drugs, prolonging the 1/2t,

- Long term use of pPB-HSA requires a sophisticated formulation, such as polymeric microspheres for controlled and sustained release.

Aim

The aim of this research was to develop a solid formulation for the controlled and sustained release of pPB-HSA and assess the delivery and targeting of the intact protein construct in vivo.

Methods – formulation development

Microsphere production - Water-in-oil-in-water process

- 1.0 g microsphere batches with 5% protein content were prepared using a double emulsion evaporation production process. After filtration and washing, the microspheres were freeze dried.

Polymers

- Combination of 2 polymers provides flexibility for release profile
- Semi-crystalline block copolymers developed by InnoCore Pharmaceuticals (Groningen, The Netherlands)

Microsphere characterization

- Particle size assessment: laser diffraction and scanning electron microscopy
- Total protein content/encapsulation efficiency (EE): microspheres were dissolved in a mixture of DMSO and 0.05N NaOH, 0.5% SDS. The total protein content was determined using the BCA assay.
- In vitro release: microspheres were immersed in phosphate buffer (pH 7.4) and placed in a 37°C shaking water bath. Samples were taken at predetermined time points and replaced by fresh buffer. The total protein content was measured using BCA. The microsphere content was determined using a sandwich ELISA.

Methods – in vivo experiment

Unilateral ureteral obstruction (UUO) renal fibrosis model in C57Bl/6 mice

- Ligation of the left ureter causes the development of renal tubulointerstitial fibrosis in 7 days
- Renal fibrosis in the UUO kidney is associated with increased PDGFβ-receptor expression

Study design

- Day 0: UUO surgery
- Day 0: Subcutaneous administration of 31.5 mg microspheres (dispersed in 0.5 ml, 0.4% carbomethoxymethylcellulose solution)
- n= 3 pPB-HSA / 2% HSA microsphere administration
- Day 0: Sacrifice of animals
- Collection of kidneys and blood for analysis

Analysis

- ELISA and western blot for pPB-HSA in plasma
- Western blot for HSA in kidney tissue
- Immunohistochemical staining on pPB-HSA in kidney sections

Conclusions

- pPB-HSA was successfully formulated in polymeric microspheres produced by a W/O/W method, which showed a first order release profile in vitro for 14 days.
- pPB-HSA was released from these microspheres in vivo.
- 7 days after administration, pPB-HSA was detectable in plasma and predominantly localized in fibrotic tissue with increased expression of the target, the PDGFβ-receptor.

The delivery and site specific targeting of pPB-HSA from polymeric microspheres is feasible and opens opportunities for developing controlled release formulations with therapeutic proteins targeted to fibrotic tissue.

Results – formulation development

Microsphere size and appearance

- After 7 days of in vitro release, small pores are formed, but no substantial degradation is visible.
- The particle size distributions of the two microsphere batches are comparable and confirm the SEM photographs.

Table: Particle size distribution

<table>
<thead>
<tr>
<th>Protein</th>
<th>X1 (μm)</th>
<th>X2 (μm)</th>
<th>X6 (μm)</th>
<th>Encapsulation Efficiency</th>
<th>Recovery after 140 release</th>
</tr>
</thead>
<tbody>
<tr>
<td>pPB-HSA/HSA</td>
<td>6.55</td>
<td>24.74</td>
<td>50.22</td>
<td>83%</td>
<td>95%</td>
</tr>
<tr>
<td>HSA</td>
<td>6.58</td>
<td>26.36</td>
<td>55.79</td>
<td>81%</td>
<td>105%</td>
</tr>
</tbody>
</table>

50:50 polymer composition shows most suitable release profile

- Release accelerates with increasing content of polymer 2.
- 50:50 ratio is an exception: this formulation shows the fastest release.
- The burst release of all ratios is negligible.

In vitro release of microspheres for in vivo experiment

- The in vitro total protein release of the two batches is comparable. After 7 days, 80% has been released.
- The release of pPB-HSA from the microspheres shows the same profile as the total protein release.

In vitro total protein release of 5% HSA and 3% pPB-HSA / 2% HSA microspheres

Results – in vivo experiment

Microspheres after subcutaneous administration

- The pPB-HSA was administered subcutaneously, indicating that the release was still ongoing.

pPB-HSA in plasma of UUO mice

- 7 days after administration, HSA and pPB-HSA were still present in microspheres that were localized subcutaneously, indicating that the release was still ongoing.

pPB-HSA concentration in plasma

- 7 days after administration, pPB-HSA was present in plasma of pPB-HSA treated mice, as shown by ELISA and western blot.

pPB-HSA detection in the target organ: fibrotic kidney

Western blot analysis convincingly shows specific targeting of released pPB-HSA to the fibrotic kidney. Moreover, the leakage of HSA to the fibrotic kidney is not significant.

Collagen deposition is increased in UUO kidneys 7 days after ligation. pPB-HSA is present in UUO kidney tissue of pPB-HSA treated mice.

Collagen deposition increased in UUO kidneys 7 days after ligation. pPB-HSA is present in UUO kidney tissue of pPB-HSA treated mice.

Legend

- Western blot analysis
- Colleague 1 and lab marking