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Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Biodegradable microspheres for the sustained release of pPB-HSA to target PDGFB-receptors in fibrotic tissues

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Methods

Introduction

Polymer
• Microsphere characterization formulation for the controlled and sustained release of PDGF development of fibrotic processes in several tissues. Accordingly, the Platelet Analysis unilateral ureteral obstruction (UUO) renal fibrosis model in C57Bl6 mice

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 0.05% 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 pPB-HSA content was determined using a sandwich ELISA.

Methods – in vivo experiment

Unilateral ureteral obstruction (UUO) renal fibrosis model in C57Bl6 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 PDGFB-receptor expression

Study design
• Day 0: UUO surgery
• Day 0: Subcutaneous administration of 31.5 mg microspheres (dispersed in 0.5 mL 10% carbomethylcellulose solution)
• n= 5 HSA microsphere administration
• n= 3 pPB-HSA / 2% HSA microsphere administration
• Day 4: 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 PDGFB-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.

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.

Results – in vivo experiment

In vitro total protein release of 5% HSA and 3% pPB-HSA / 2% HSA microspheres
• The burst release of all ratios is negligible.

In vitro release of 5% HSA microspheres with different polymer ratios
50:50 polymer composition shows most suitable release profile

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

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.

pPB-HSA is present in UUO kidney tissue of pPB-HSA treated mice.

Notes:
1. Funding by SNN
2. The data presented in this poster was prepared in collaboration with InnoCore Pharmaceuticals.