Development of a Therapeutic Cancer Vaccine based on Polymeric Nanoparticles containing HPV Synthetic Long Peptide and Poly IC

Sima Rahimian¹, Marieke F. Fransen², Jan Willem Kleinovink², Jonathan Christensen¹, Maryam Amidi¹, Ferry Ossendorp² and Wim E. Hennink¹

¹Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, 3584CG Utrecht, The Netherlands.
²Department of Immunohematology and Blood Transfusion, Leiden University Medical Center; 2333 ZA Leiden, The Netherlands
S.Rahimian@uu.nl

ABSTRACT SUMMARY
We report a promising approach towards design of a cancer vaccine formulation for treatment of Human papillomavirus (HPV)-induced cervical cancer. Polymeric nanoparticles containing a synthetic long peptide derived from HPV16 oncoprotein and a Toll like receptor 3 (TLR3) ligand (polyIC) were prepared and characterized for their ability to induce specific T cell immunity in mice.

INTRODUCTION
Immunotherapy of cancer has been established as a groundbreaking approach to treat malignancies. Conventional cancer treatment strategies employ methods to eliminate all rapidly proliferating cells. Immunotherapy, however, involves targeting the immune system with specific molecules to fight cancer. Therapeutic vaccination is among the most promising modalities of immunotherapy. An effective cancer vaccine is capable of activating the adaptive as well as the innate immune system, resulting in efficient targeting and elimination of cancer cells through a strong T cell-mediated immune response. Of several cancer immunization approaches, vaccination with synthetic long peptides (SLPs) has shown encouraging results. In a recent phase I/II clinical trial, human papillomavirus (HPV)–induced cervical cancer patients were vaccinated with SLPs derived from specific HPV16 oncoproteins formulated in incomplete freund’s adjuvant (IFA). SLPs were capable of inducing strong specific T cell immunity¹, though the use of IFA was associated with adverse effects such as swelling and fever². Furthermore it has been shown that introducing adjuvants such as TLR ligands to cancer vaccine formulations results in enhanced antigen cross presentation and specific CD8⁺ T cell response. In order to increase the efficacy and safety of HPV SLP cancer vaccine, we have developed a nanoparticle (NP) formulation using a biodegradable polymer (polylactide-co-hydroxymethylglycolide (pLHMGA)). Previous studies have shown the advantages of this polymer over the well known and frequently investigated pLGA, such as better compatibility with proteins because of presence of hydrophilic functional groups, which additionally provide the possibility to conjugate targeting ligands to the polymer³. Our aim is to develop a cancer vaccine formulation comprised of a synthetic long peptide containing CD8⁺ epitope of HPV (HPV SLP) as well as a TLR3 ligand (Poly IC) as an adjuvant and study the effect of encapsulation of antigens and adjuvants in polymeric nanoparticles on T cell response and their potential in immunotherapy of cancer.

EXPERIMENTAL METHODS
pLHMGA nanoparticles loaded with HPV SLP, poly IC alone or co-encapsulated were prepared by double emulsion solvent
evaporation technique. In a typical procedure, 200 µl of 10 mg/ml HPV SLP in acetonitrile/TFA 0.1% in water (1:1) and 50 µl of 20 mg/ml Poly IC were emulsified in 1 ml of dichloromethane containing 10% pLHMGA, this primary emulsion was subsequently emulsified in aqueous solution of PVA 1% w/v and then transferred into 25 ml of PVA 0.3% w/v. After evaporation of DCM, the particles were washed with endotoxin-free water and freeze dried. Particle size and morphology were characterized by dynamic light scattering and transmission electron microscopy. Loading efficiency of HPV SLP in the particles was measured by reverse phase HPLC and the amount of Poly IC encapsulated was determined by Quantifluor RNA quantification assay. For evaluation of endogenous T cell expansion in vivo, combinations of nanoparticle formulations with or without soluble Poly IC were subcutaneously administered into mice and 7 days later the level of HPV specific CD8⁺ T cells in the systemic circulation was analyzed using a tetramer staining assay. Soluble HPV SLP plus Poly IC and HPV SLP in incomplete freund’s adjuvant (IFA) were used as controls. The therapeutic efficacy of the nanoparticle vaccines in tumor bearing mice is under evaluation.

RESULTS AND DISCUSSION

Spherical nanoparticles were obtained with a size ranging from 400-500 nm. Loading efficiency of HPV and poly IC in the nanoparticles were around 50%. Characteristics of the obtained nanoparticles are summarized in Table 1.

Table 1 - Characteristics of nanoparticles.

<table>
<thead>
<tr>
<th>Nanoparticles (NP)</th>
<th>Size (nm)</th>
<th>HPV loading efficiency (%)</th>
<th>Poly IC loading efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pLHMGA HPV</td>
<td>459-0.24</td>
<td>52.4</td>
<td>-</td>
</tr>
<tr>
<td>pLHMGA HPV + poly IC</td>
<td>507-0.36</td>
<td>52.0</td>
<td>52.4</td>
</tr>
<tr>
<td>pLHMGA poly IC</td>
<td>413-0.40</td>
<td>-</td>
<td>53.9</td>
</tr>
</tbody>
</table>

HPV specific CD8⁺ T cell were readily detectable in mice immunized with any combination of HPV NP and poly IC, regardless of poly IC being in the same particle (co-encapsulated) or being in separate particles or even in soluble form, while HPV SLP in IFA and a combination of HPV SLP and Poly IC exhibited significantly lower levels of specific T cell in blood.

CONCLUSION

This study shows that encapsulation of HPV SLP antigen in pLHMGA nanoparticles substantially enhances the population of specific T cell when combined with a TLR3 ligand. This suggests that pLHMGA nanoparticles are a suitable candidate for delivery of HPV SLP tumor-antigen.

REFERENCES


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