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Human serum albumin nanoparticles for ocular delivery of bevacizumab

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ABSTRACT

Bevacizumab-loaded nanoparticles (B-NP) were prepared by a desolvation process followed by freeze-drying, without any chemical, physical or enzymatic cross-linkage. Compared with typical HSA nanoparticles cross-linked with glutaraldehyde (B-NP-GLU), B-NP displayed a significantly higher mean size (310 nm vs. 180 nm) and a lower negative zeta potential (−15 mV vs. −36 mV). On the contrary, B-NP displayed a high payload of approximately 13% when measured by a specific ELISA, whereas B-NP-GLU presented a very low bevacizumab loading (0.1 μg/mg). These results could be related to the inactivation of bevacizumab after reacting with glutaraldehyde. From B-NP, bevacizumab was released following an initial burst effect, proceeded by a continuous release of bevacizumab at a rate of 6 μg/h. Cytotoxicity studies in ARPE cells were carried out at a single dose up to 72 h and with repeated doses over a 5-day period. Neither bevacizumab nor B-NP altered cell viability even when repeated doses were used. Finally, B-NP were labeled with ^{99m}Tc and administered as eye drops in rats. ^{99m}Tc-B-NP remained in the eye for at least 4 h while ^{99m}Tc-HSA was rapidly drained from the administration point. In summary, HSA nanoparticles may be an appropriate candidate for ocular delivery of bevacizumab.

1. Introduction

Human serum albumin (HSA) is the most abundant protein in human blood, and is a natural and adequate material for the fabrication of nanoparticles for drug-delivery purposes. In recent decades albumin nanoparticles have gained considerable attention owing to their high capability to load a number of drugs in a non-specific way (Ghuman et al., 2005), as well as their tolerability when administered in vivo (Green et al., 2006). For the preparation of these nanocarriers a great variety of physico-chemical processes have been proposed, including thermal gelation (Qi et al., 2010; Yu et al., 2006a,b), emulsification (Crisante et al., 2009; Patil, 2003; Yang et al., 2007) and desolvation (coacervation) (Merodio et al., 2001; Weber et al., 2000; Wilson et al., 2012). In any case, desolvation-based procedures appear to be most suitable due to their simplicity and repeatability. However, the just obtained albumin nanoparticles are unstable and a supplementary step of stabilization or cross-linkage has to be performed in order to prolong their half-life in an aqueous environment and/or prevent the formation of macro-aggregates of the protein.

In general, cross-linkage with glutaraldehyde (GLU) is one of the most frequently implemented strategies to stabilize albumin nanoparticles. While it is highly effective for this purpose, the use of GLU (and other derivatives) is questionable due mainly to its toxicity and reactivity against some functional groups (e.g. primary amine residues) (McGregor et al., 2006; Van Miller et al., 2002). Thus, for the delivery of biomacromolecules (e.g. antibodies, proteins, peptides), this dialdehyde may also react with the biologically-active compound, resulting in an important loss of their activity and efficacy (Zhang et al., 2008). In addition, the potential toxicity of GLU is a concern for in vivo delivery (McGregor et al., 2006; Van Miller et al., 2002; Zhang et al., 2008). In order to overcome these drawbacks, different strategies have been proposed to harden the just formed albumin nanoparticles without the need of using toxic reagents. Amongst others, this stabilization of nanoparticles from proteins can be obtained through thermal treatment (Yang et al., 2007), high hydrodynamic pressure (Desai, 2006), enzymatic cross-linkage with genipin (Elzoghby et al., 2013; Kang et al., 2003) or transglutaminase (Huppertz and de Kruif, 2008).

Bevacizumab is a G immunoglobulin (MW of 149 kDa) composed of two 214-residue light chains and two 453-residue heavy chains that contain an N-linked oligosaccharide. This monoclonal antibody,

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