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Design of targeted lipid nanocapsules by conjugation of whole antibodies and antibody Fab' fragments

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Abstract

Immunonanocapsules were synthesized by conjugation to lipid nanocapsules (LNC) of whole OX26 monoclonal antibodies (OX26 MAb) directed against the transferrin receptor (TfR). The TfR is overexpressed on the cerebral endothelium and mediates the transcytosis mechanism. Fab' fragments, known for their reduced interaction with the reticuloendothelial system, were also conjugated to LNC. This coupling was facilitated by the incorporation of lipid PEG_{2000} functionalized with reactive-sulfhydryl maleimide groups (DSPE–PEG_{2000}–maleimide) into LNC shells by a post-insertion procedure, developed initially for liposome pegylation. An interfacial model using the dynamic rising drop technique helped determine the parameters influencing the DSPE–PEG_{2000}–maleimide insertion and the quality of the anchorage. Heat was essential to promote both an important and stable adsorption of DSPE–PEG_{2000}–maleimide onto LNC. OX26 MAb were thiolated to react with maleimide functions whereas thiol residues on Fab' fragments were used directly. The number of ligands per nanocapsule was adjusted according to their initial quantity in the coupling reaction mixture, with densities from 16 to183 whole antibodies and between 42 and 173 Fab' fragments per LNC. The specific association of immunonanocapsules to cells overexpressing TfR was thus demonstrated, suggesting their ability to deliver drugs to the brain.

Keywords: Immunonanocapsules; Ox26 monoclonal antibody; Fab' fragment; Transferrin receptor; Brain targeting; Interfacial rheology

1. Introduction

Colloidal drug carriers such as liposomes and nanoparticles have been widely used for systemic drug delivery. Packaged into a nanocontainer, the drug is protected from chemical and enzymatic degradation. The first generation of nanocarriers, was developed 40 years ago. However, the carriers were rapidly eliminated from the bloodstream by the reticuloendothelial system (RES). Consequently, drug delivery could only reach the liver and spleen. In order to improve the vascular residence time of colloidal systems, hydrophilic and flexible polymers such as poly(ethylene) glycol (PEG) were grafted onto their surface, thus conferring steric protection [1]. Their main therapeutic application concerns drug delivery to tumor sites by way of the enhanced permeability and retention effect (EPR effect) [2].

Lipid nanocapsules (LNC) [3] belong to this generation of stealth nanovectors. These colloidal carriers, in the nanometer size range, are produced using a solvent-free process with biocompatible excipients. They are made up of an oily core surrounded by a hydrophilic surfactant, Solutol® HS15 (70% PEG_{660} hydroxystearate and 30% free PEG_{660}) conferring long-circulating properties [4] and inhibiting the P-glycoprotein efflux pump (P-gp) [5,6]. LNC can be loaded with anti-cancer agents such as etoposide and paclitaxel and can thus efficiently deliver drugs to glioma cells [7,8].

As with most stealth nanocarriers, the therapeutic limitation of LNC is due to their non-specificity and their inability to cross the weakly permeable endothelia. Thus,

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these nanovectors cannot be used for drug delivery to the central nervous system (CNS). The blood brain barrier (BBB), separating the blood and the cerebral parenchyma, is mainly composed of brain capillary endothelial cells (BCEC) sealed together by continuous, tight junctions, drastically decreasing its permeability. Given this context, the LNC used for active targeting were modified using site-specific ligands to allow drug delivery to the CNS. OX26 murine monoclonal antibodies (OX26 MAb) that recognize the transferrin receptor (TfR) were conjugated to LNC (OX26-immunonanocapsules). This antibody specifically targets the BCEC through a high concentration of TfR expressed on its luminal side [9] and is able to cross the BBB via a receptor-mediated transcytosis mechanism [10]. OX26 MAb binds to an extracellular domain of the TfR, distinct from the transferrin binding site, thus avoiding competition with the endogenous transferrin in the circulation system. Besides, the Fc part of the whole antibody is known to activate both the usual pathway of the complement system and the macrophage-bearing Fc receptors on their surface. Consequently, the conjugation of entire antibodies may significantly decrease the vascular residence time of immunonanocapsules [11]. Thus, OX26 MAb Fab' fragments characterized by the absence of the Fc part, were also conjugated to LNC (Fab'-immunonanocapsules).

The feasibility and potential of this active targeting strategy using nanocarriers have already been demonstrated. OX26-immunoliposomes are promising novel vectors for the delivery of doxorubicin and daunomycine to the brain [12,13]. OX26-PLA and OX26-chitosan nanoparticles [14,15] were conceived successfully and the chitosan immunonanoparticles were able to reach the cerebral parenchyma.

To allow the covalent attachment of site-directed biomolecules, a bifunctional polymer, distearoylphosphatidylethanolamine–PEG_{2000}–maleimide (DSPE–PEG_{2000}–maleimide) was incorporated into the LNC shell. Maleimide functions react with sulfhydryl groups to form thioether bonds. In contrast to Fab' fragments which bear thiol residues on the intra-heavy chain, this reaction requires the thiolation of OX26 MAb. Besides, the incorporation of DSPE–PEG_{2000}–maleimide was performed using a post-insertion procedure. This method was initially developed for the pegylation of liposomes. It consists in incubating particles in a micellar solution of PEG lipids. During incubation, a temperature-induced transfer occurs between phospholipids located in the liposomal system and lipid-conjugated polymers. Uster et al. [16] demonstrated that the incorporation rate of PEG_{1900}–DSPE into liposomes was close to the optimum value after a 1 h incubation period at 60 C.

This present work describes the synthesis of immunonanocapsules.

Firstly, incorporation of the DSPE–PEG_{2000}–maleimide into an LNC shell was characterized using an interfacial model. The effects of both temperature and the interactions between the bifunctional polymer and the components located at the LNC surface were studied. As for liposomes, the presence of phospholipids at the interface was presumed necessary in order to facilitate the transfer of matter. Conversely, the presence of Solutol® HS15 generating a steric barrier around the nanocapsule could reduce the DSPE–PEG_{2000}–maleimide insertion. The polymer was adsorbed at pure O/W interface and on interfacial monolayers composed of Lipoïd® S75-3 or/and Solutol® HS15, using a drop tensiometer. The superficial pressures were measured at 25 and 60 °C for a 2 h-adsorption period and after cooling from 60 to 25 °C. The rheological behavior of these films was then determined using the generalized Maxwell model in order to identify the quality of the bifunctional polymer anchorage. This was to help determine whether or not the DSPE–PEG_{2000}–maleimide could bear whole OX26 MAb and Fab' fragments without desorption.

Secondly, we developed the conjugation of biomolecules on LNC. Thiolation and fragmentation of MAb were performed to covalently attach antibodies and Fab' fragments onto the LNC surface. The influence of this chemical treatment on the recognition activity of biomolecules was studied by flow cytometry. The immunonanocapsules were then characterized by assessing the ligand density and measuring the size. Finally, their ability to target cells overexpressing TfR was verified by flow cytometry.

2. Materials and methods

2.1. Reagents

Lipoïd® S75-3 (soybean lecithin at 69% of phosphatidylcholine and 10% phosphatidylethanolamine) and the nonionic hydrophilic surfactant Solutol® HS15 were kindly supplied by Lipoïd GmbH (Ludwigshafen, DE) and BASF (Ludwigshafen, DE), respectively. The lipophilic Labrafac® WL 1349 (caprylic–capric acid triglycerides) was generously provided by Gattefossé S.A. (Saint-Priest, FR). Due to the complex composition of these products, they will henceforth be referred to by their brand names (Lipoïd®, Solutol® and Labrafac®). NaCl was purchased from Prolabo (Fontenay-sous-bois, FR). Deionised water was obtained from a Milli-Q plus® system (Millipore, Billerica, USA). DSPE–PEG_{2000}–maleimide was purchased from Avanti Polar Lipids (Alabaster, USA). $Na^{125}I$ and HiTrap® protein high performance columns were provided by Pharmacia-Biotech (Uppsala, SE), Sepharose CL-4B, dithiotreitol (DTT), sodium *meta*-periodate, Ellman's reagent, 2-mercaptoethylamine hydrochloride (MEA · HCl), Sephadex G-25 and G-50 were obtained from Sigma (Saint-Louis, USA). The PDPH [3-(2-pyridyldithio)-propionyl hydrazide], the "ImmunoPure $F(ab')_2$ Preparation Kit" and Iodo-Gen Reagent (1,3,4,6-tetrachloro-3α-6α-diphenylglycouril) were purchased from PIERCE (Rockford, USA). The fluorescein isothiocyanate (FITC)-conjugated goat $F(ab')_2$ anti-mouse immunoglobulin (IgG) was provided by Dako (Glostrup, DK) and the mouse isotype control IgG2a was from BD Pharmingen (San Jose, USA).

2.2. Cells

The OX26 and Y3.AG.1.2.3. hybridoma cell lines were supplied by Canada Research Chair in Drug Delivery (Montreal, CAN). OX26 hybridoma was grown as a suspension culture in RPMI 1640 medium