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Research paper

A quantitative evaluation of the molecular binding affinity between a monoclonal antibody conjugated to a nanoparticle and an antigen by surface plasmon resonance

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ABSTRACT

We have designed a site-specific drug colloidal carrier ultimately for improving pancreatic and lung cancer treatment. It is based on a nanoparticulate drug delivery system that targets tumors overexpressing H-ferritin. A monoclonal antibody, AMB8LK, specifically recognizing H-ferritin was thiolated and conjugated to maleimide-activated polylactide nanoparticles (NPs) resulting in the formation of immunonanoparticles (immunoNPs). The AMB8LK immunoNPs exhibited a mean diameter size of 112 ± 20 nm and a density of 76 antibody molecules per NP. AMB8LK immunoNPs were evaluated for uptake and binding properties on CAPAN-1 and A-549 cell lines, using confocal microscopy. ImmunoNPs demonstrated specific binding and increased uptake of the desired cells by means of monoclonal antibodies (MAbs), compared to nonconjugated NPs. A lipophilic paclitaxel derivative, paclitaxel palmitate (pcpl), was encapsulated within the various NP formulations, and their cytotoxic effect was evaluated on A-549 cells using MTT assay. Pcpl-loaded AMB8LK immunoNPs showed a significantly increased cytotoxic effect when compared to pcpl solution and pcpl NPs. Surface plasmon resonance (SPR) was used to determine quantitatively the affinity constants of native AMB8LK and AMB8LK immunoNPs to gain insight on the affinity of the MAbs following the conjugation process onto NPs. The results of the association/dissociation and affinity kinetics of the interaction between H-ferritin and native AMB8LK or AMB8LK immunoNPs revealed similar constant values, showing that the conjugation process of the MAb to the NPs did not alter the intrinsic specificity and affinity of the MAb to the antigen. In conclusion, at the cellular level, AMB8LK immunoNPs may carry drugs to desired overexpressing antigen cells with adequate affinity properties, potentially leading to improved drug therapy and reduced systemic adverse effects.

1. Introduction

Active targeting of nanoparticulate drug delivery systems has raised interest for improved cancer therapy. Monoclonal antibodies (MAbs) have been the most widely investigated targeting moieties following their conjugation to nanoparticles (NPs), which results in the formation of immunonanoparticles (immunoNPs) [1,2]. ImmunoNPs comprised of pegylated polyesters are considered to be the most suitable nanocarriers of chemotherapeutic agents to the tissue of interest, primarily due to their long circulating time [3,4], biodegradability, biocompatibility [5,6] and high drug payloads [7,8]. In addition to all these significant features, immunoNPs possess adequate physicochemical shelf life over long-term storage as freeze-dried powders, which can maintain their initial properties upon reconstitution with the addition of sterile water prior to use [9,10]. In previous reports, the physicochemical properties of drug-loaded immunoNPs have been evaluated in terms of type of covalent binding between the MAb to the NP, amount of MAbs attached to the NPs, recognition properties of immunoNPs on cultured cell lines and their ability to enhance cell internalization [11,12]. Recently, we designed polylactide (PLA) NPs loaded with the anti-cancer agent paclitaxel palmitate that were conjugated to an anti-HER-2 receptor MAb, trastuzumab [13]. These immunoNPs exhibited relatively high payloads of drug, excellent binding properties and significant cell uptake to HER-2 overexpressing cells. It was further observed in healthy mice that the pharmacokinetic behavior of the immunoNPs was markedly different from the pharmacokinetic profile of the naked MAb, demonstrating that the MAb lost its intrinsic

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molecular pharmacokinetic properties following conjugation to the NPs. The immunoNPs elicited a significant anti-tumor activity in an orthotopic human prostate tumor induced in mice, when compared to the pcpl solution and NPs, although the tumor growth was not fully inhibited [13]. Despite these encouraging results, there was no accurate information on the damage elicited to the MAb following its conjugation to the NPs. It was then decided to carry out a quantitative investigation of the association and dissociation kinetics of immunoNPs to their antigen, when compared to the native MAb. For such a purpose, we applied the surface plasmon resonance (SPR) technique that has been used mainly in protein–ligand and antibody–antigen interaction studies. Although molecular interactions of antibody–antigen per se are well-established following numerous comprehensive affinities and kinetic analyses using SPR technique [14–16], they are not in the scope of the present manuscript. SPR allows real time analysis of molecular association between a MAb and an antigen and subsequent kinetic characteristics derived from the changes in refractive index close to a metal surface on which the antigen or the MAb is attached. When a molecule from the solution binds to the immobilized molecule on the metal surface, the resonance angle changes, and the response is recorded in a resonance unit (RU) [17,18]. Moreover, SPR has been used to investigate metal NPs for chemical sensing, imaging and targeting [19–21]. As an example, folic acid as a targeting moiety was conjugated to metal NPs and, its specific recognition by folic acid binding protein was demonstrated using SPR [22]. However, metal NPs cannot easily be used as drug delivery systems. It should be emphasized that SPR has been used to evaluate both qualitatively and quantitatively the affinity of antibodies attached to liposomes towards various antigens or receptors overexpressed in pathological tissues [23,24]. Furthermore, Kocbek and Coll. carried out a SPR study with anti-cytokeratin MAb attached covalently to NPs and evaluated the interaction of the immunoNPs with protein A [25]. The selected MAb in the present investigation is AMB8LK, which exhibits marked affinity for specific tumor organs overexpressing H-Ferritin [26]. It was previously demonstrated that total ferritin increased and shifted toward acidic (H-rich) ferritin [27] in the serum of patients with various malignancies such as colon cancer [28], testicular seminoma [29], breast cancer [30] and pancreatic cancer [31]. AMB8LK has been shown to exhibit marked affinity for specific tumor organs such as pancreatic and lung (NSCLC) cancers [32]. Moreover, a polyclonal antibody recognizing H-ferritin following coupling with yttrium 90 (Ferritarg P®) is currently under clinical evaluation in patients suffering relapsed or refractory Hodgkin's disease [33]. In this study, we have prepared and characterized immunoNPs conjugated to the anti-human H-ferritin AMB8LK MAb with or without the anti-cancer agent, paclitaxel palmitate. These immunoNPs were then evaluated on H-ferritin overexpressing cells for uptake, binding properties and cytotoxic effects. In the second part of this study, we have used SPR detection to monitor the reaction between these immunoNPs and their antigen, human H-ferritin. Here, we present a quantitative determination of immunoNPs' association and dissociation kinetics to their antigen when compared to the naked MAb.

2. Experimental

2.1. Materials

The polymer poly(ethylene glycol-co-lactide) MW 100,000 (PEG–PLA) was synthesized using the ring-opening polymerization method in the presence of stannous 2-ethylhexanoate as catalyst [34] and was described earlier [13].

Paclitaxel was purchased from Asia Talent Chemical, Shenzhen, China. Coumarin-6, Dimethyl sulfoxide (DMSO), 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT), 9-diethylamino-5H-benzo[α]phenoxazine-5-one (Nile Red) and polysorbate 80 (Tween® 80) were acquired from Sigma (St. Louis, MO, USA). Macrogol 15 hydroxystearate (Solutol® HS 15) was purchased from BASF (Ludwigshafen, Germany). FITC-labeled goat anti-mouse IgG and goat anti-mouse PE-labeled secondary antibodies were obtained from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Human Transferrin, Texas Red conjugated/tagged was obtained from Molecular Probes (Eugene, OR, USA). All organic solvents were HPLC grade and purchased from J.T. Baker (Deventer, Holland).

A research-grade CM5 (carboxymethyl dextran) sensor chip, NHS (*N*-hydroxysuccinimide), EDC [*N*-ethyl-*N*_-(3-dimethylaminopropyl)-carbodi-imide hydrochloride], ethanolamine/HCl and HBS-EP running buffer (10 mM Hepes, 150 mM NaCl, 3.4 mM EDTA and 0.005%, v/v, surfactant P20 at pH 7.4) were purchased from Biacore AB (Uppsala, Sweden). Human H-ferritin was obtained from Scripts (San Diego, CA, USA), while its MAb AMB8LK was a kind gift from MAT Biopharma (Evry, France). Human transferrin was purchased from Gibco (Invitrogen, Rockville, MD, USA).

2.2. Cell cultures

CAPAN-1 cell line (American Type Culture Collection, Rockville, MD, USA) was cultured in DMEM plus 20% fetal calf serum, 1% non-essential amino acids, 1% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were maintained at 37 °C under 5% CO_2. A-549 cells (American Type Culture Collection, Rockville, MD) were grown in F-12K medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were maintained at 37 °C under 5% CO_2. All cell culture products were obtained from Biological Industries (Kibbutz Beit Haemek, Israel).

2.3. Methods

2.3.1. Preparation of nonconjugated NPs or pcpl-loaded AMB8LK immunoNPs

The synthesis of pcpl [35] and the preparation of NPs and immunoNPs [13] were described in detail previously. In brief, mPEG–PLA MW 100,000 at a concentration of 0.6% w/v and the linker OMCCA at a concentration of 0.04% w/v were dissolved in 50 ml acetone containing 0.2% w/v Tween® 80. If pcpl-loaded immunoNPs were prepared, then 0.08% w/v of pcpl was added and dissolved into the organic phase. The organic phase was added to 100 ml of the aqueous phase which contained 0.1% w/v Solutol® HS 15. The suspension was stirred at 900 rpm over 1 h and then concentrated by evaporation to 10 ml. The formulations were adjusted to pH 8 and incubated overnight at 4 °C under nitrogen with thiolated AMB8LK MAb. The formulation was diafiltrated with 100 ml solution of 0.1% Tween® 80 (Vivaspin 300,000 MWCO, Vivascience, Stonehouse, UK) and filtered through 1.2 μm filters (FP 30/1.2 CA, Schleicher & Schuell, Dassel, Germany). When fluorescent NPs were needed, coumarin-6 was added to the organic phase resulting in a final concentration of 3 μg/ml.

2.3.2. AMB8LK immunoNP characterization

2.3.2.1. Particle size analysis. Mean diameter measurements of three batches of the same formulation was carried out utilizing an ALV Noninvasive Back Scattering High Performance Particle Sizer (ALV-NIBS HPPS, Langen, Germany) at 25 °C and using water as diluent. The sensitivity range was 0.5 nm to 5 μm.