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Targeted delivery of anti-CD19 liposomal doxorubicin in B-cell lymphoma: A comparison of whole monoclonal antibody, Fab′ fragments and single chain Fv

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Abstract

As part of an ongoing effort to develop a clinically acceptable doxorubicin formulation, targeted against B-cell malignancies, this study compared long-circulating (Stealth®) immunoliposomes (SIL) that were targeted against the B-cell antigen CD19, via a whole HD37 monoclonal antibody (HD37 mAb), versus its Fab′ fragment (HD37 Fab′) or an HD37–c-myc–Cys–His5 single chain Fv fragment (scFv, HD37-CCH) directed against the same epitope. Compared to untargeted liposomes (SL), SIL showed increased binding in vitro to CD19-expressing Raji cells and, when loaded with doxorubicin (SIL–DXR), increased cytotoxicity against Raji ($CD19^+$), but not Molt4 ($CD19^-$) cells. Pharmacokinetics and biodistribution studies using dual-labeled liposomes (lipid and drug) in naïve and Raji-bearing mice showed that SIL–DXR targeted via HD37 Fab′ exhibited the same long circulation half-life as SL–DXR. SIL–DXR targeted via HD37-CCH was cleared faster than SL–DXR due to increased liver uptake, which was related to the poly-His and/or the c-myc tags in the scFv construct. SIL–DXR targeted via HD37 mAb was cleared rapidly from circulation due to Fc-mediated uptake in the liver and spleen. All three formulations of SIL–DXR extended the mean survival time of Raji-bearing mice compared to SL–DXR or free DXR. SIL–DXR targeted via HD37 Fab′, which had the longest circulation half-life, appeared to be slightly more effective in prolonging survival times than SIL–DXR targeted via either HD37-CCH or HD37 mAb.

Keywords: Anti-CD19; scFv; Liposomes; Doxorubicin; B-lymphoid cells; Immunoliposomes

1. Introduction

Conventional anticancer drugs have many adverse effects resulting from non-selective toxicity and distribution of the drug to normal cells. Liposomes were one of the first nanoparticulate drug delivery systems to show increased delivery of small molecule anticancer drugs to solid tumors [1,2]. Drug-loaded liposomes with diameters in the range of 100 nm can accumulate in solid tumors via the enhanced permeability and retention (EPR) effect [3,4], which occurs when nanoparticles extravasate from the circulation into tumors through gaps in the tumor vasculature endothelium [5]. Once there, the liposomes release their contents at a rate that is determined by the physical properties of the liposome and the drug [6]. The ability of liposomes to localize in tumors via the EPR effect depends in part on their having long circulation half-lives ($T_{1/2}$ on the order or 24 h or longer), which can be achieved by grafting polyethylene glycol (PEG) to the surface of the liposomes (i.e., Stealth® liposomes, SL). A Stealth® (PEGylated) liposomal formulation of the anticancer drug doxorubicin (DXR), Doxil/Caelyx®, which is approved in the treatment of Kaposi's cancer and ovarian cancer, has been in clinical use for over a decade [7,8].

Stealth® liposomal drugs can be specifically targeted to cancer cells by the surface conjugation of one or more of a variety of ligands against tumor antigens that are uniquely expressed or over-expressed on the tumor cells. Ligands used for targeting include whole monoclonal antibodies (mAb) or their fragments, e.g., Fab′ or single chain Fv (scFv) [9,10].

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Although no immunoliposomes are yet in clinical use, extensive pre-clinical research activity is taking place in this area.

Previously published studies from this laboratory have shown that treatment of murine models of human B lymphoma with Stealth® immunoliposomal doxorubicin (SIL–DXR), targeted via the FMC63 anti-CD19 mAb or its Fab′ fragment, resulted in increased survival times relative to free DXR or untargeted Stealth® liposomal DXR (SL–DXR) [11]. The CD19 antigen is an attractive target for delivery of liposomal anticancer drugs since it is an internalizing antigen exclusive to B cells that is expressed in most types of B-lymphoid malignancies [12]. Internalization of the antibody–CD19 complex and its recycling back to the cell surface from the endosomal compartment has been shown to be important for delivery of liposomal DXR [13,14].

At the pre-clinical development stage of antibody-targeted liposomes it is important to have a clear understanding of the advantages and disadvantages of the use of whole mAb vs. Fab′ or scFv fragments as targeting ligands. Factors to be considered include the ease of production, yield, ease of purification, stability, affinity and avidity, immunogenicity and pharmacokinetics/biodistribution (PK/BD) [15]. Improved circulation half-lives and therapeutic effects have been observed when SIL conjugated with anti-CD19 Fab′ fragments were compared to SIL conjugated with the parental whole mAb, since Fab′-targeted liposomes avoid Fc-mediated clearance [11,16,17]. These experiments have now been extended to include a comparison with scFv fragments, since the smaller fragments may have advantages such as further reductions in immunogenicity and ease of production. The present study compared SIL targeted via the anti-CD19 HD37 mAb, its Fab′ fragment and an scFv construct, all of which bind to the same epitope on CD19. The current study examined the *in vitro* binding and cytotoxicity, the *in vivo* pharmacokinetics and biodistribution (PK/BD), and therapeutics of all three formulations of anti-CD19 SIL, compared to untargeted liposomes.

2. Materials and methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (HSPC) and methoxy-poly(ethylene glycol) (MW 2000), covalently linked to distearoylphosphadidylethanoloamine (mPEG$_{2000}$-DSPE), were generous gifts from ALZA Corporation, Inc. (Mountain View, CA). Cholesterol (Chol) and maleimide-derivatized PEG$_{2000}$-DSPE (Mal-PEG$_{2000}$-DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL). Bio-Rad Protein Assay Reagent was purchased from Bio-Rad Laboratories (Mississauga, ON). 2-iminothiolane (Traut's Reagent), RPMI 1640 media and Protein L agarose columns were obtained from Sigma Chemical Co. (Oakville, ON). Dithiothreitol (DTT) was purchased from Fisher Scientific (Nepean, ON). Nuclepore® polycarbonate membranes were purchased from Northern Lipids (Vancouver, BC). Chol-[1,2-^{3}H-(*N*)]hexadecyl ether ([^{3}H]CHE, 1.48–2.22 TBq/mmol) was purchased from Perkin Elmer Life Sciences (Woodbridge, ON). ^{14}C-doxorubicin (^{14}C-DXR), Sephadex G-50, Sephadex G-25, Sepharose CL-4B and Aqueous Counting Scintillant (ACS) were purchased from GE Healthcare (Baie d'Urfe, PQ). ^{125}I–Sodium iodide (^{125}I–NaI, 185 MBq), Solvable™, Ultima Gold™ and ACS were purchased from Perkin Elmer Life Sciences (Woodbridge, ON). The nickel–nitrilotriacetic acid (Ni–NTA) column was purchased from Qiagen (Hilden, Germany). Immobilized Pepsin was purchased from Pierce (Rockford, IL). Penicillin–streptomycin-L-glutamine (P/S/G, 10,000 U/mL, 10,000 μg/mL, 29.2 mg/mL, respectively), and fetal bovine serum were obtained from Invitrogen (Burlington, ON). Bacto Tryptone, Bacto Yeast Extract and Bacto Agar were from BD (Sparks, MD). All other chemicals were of the available highest grade.

2.2. Cell lines, antibodies and animals

The CD19^{+} human Burkitt's lymphoma cell line (Raji) and the CD19^{-} T cell line (Molt4) were purchased from the American Type Culture Collection (Manassas, VA). The cells were cultured in suspension in RPMI 1640 media supplemented with 10% (V/V) fetal bovine serum, and 1% (V/V) P/S/G, in a humidified 37 °C incubator with a 5% CO_2 atmosphere.

The murine anti-CD19 mAb, HD37 mAb (IgG_1), was produced and purified from the supernatant of the HD37 hybridoma cell line [18], obtained from Dr. B. Doerken (Charité, University Medicine, Berlin, Germany) via Dr. E. Vitetta (University of Texas Southwestern Medical Center, Dallas, TX). F(ab′)$_2$ fragments of the HD37 mAb were produced by digestion of the mAb using Immobilized Pepsin, according to the manufacturer's protocol. The disulfide bonds in HD37 F(ab′)$_2$ were reduced with 5 mM DTT to produce the HD37 Fab′, which contains one terminal free thiol group per Fab′ molecule for use in conjugation protocols. The plasmid for the HD37–c-myc–Cys–His5 scFv (HD37-CCH) was provided courtesy of Dr. S. Kipriyanov, Affimed Therapeutics AG, Heidelberg, Germany [19]. The c-myc and His5 tags were used for identification and purification, respectively, and the cysteine residue was used for conjugation purposes. The HD37-CCH construct, as well as the HD37-Cys construct (HD37-C), which does not contain any tag or marker, were expressed and purified from the periplasmic space of *E. coli* as previously described [15]. Briefly, scFv constructs were expressed using the pSKK vector in RV 308 *E. coli* in shaker cultures. Expression of the scFv was induced with 0.2 mM of IPTG at 22 °C when optical density at 600 nm (OD_{600}) reached 0.8. Cells were harvested by centrifugation after 16 h of induction. ScFv were extracted from the periplasmic space of *E. coli.* For HD37-CCH, scFv were purified from the periplasmic extract via affinity chromatography using a Ni–NTA column. For HD37-C, scFv were purified from the periplasmic extract using a Protein L column.

Six to eight weeks old female BALB/c CR Alt BM (BALB/c) mice were purchased from Health Sciences Laboratory Animal Services (HSLAS, University of Alberta, Edmonton, AB) and were kept in standard housing. Female 6 to 8 weeks old ICR severe compromised immunodeficient (SCID) mice were purchased from Taconic Farms (Germantown, NY) and were kept in the virus antigen-free housing of HSLAS. All