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Anti-CD30 antibody conjugated liposomal doxorubicin with significantly improved therapeutic efficacy against anaplastic large cell lymphoma

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ABSTRACT

The use of nano-carriers has been shown to improve the delivery and efficacy of chemotherapeutic agents in cancer patients. Recent studies suggest that decoration of the surface of nano-carriers with various targeting moieties may further improve the overall therapeutic efficacy. In this study, we compared the therapeutic efficacy of Doxil® (commercial doxorubicin-loaded liposomes) and that of Doxil® conjugated with anti-CD30 antibodies (CD30-targeted Doxil®) in treating anaplastic large cell lymphoma (ALCL), a type of T-cell lymphoma characterized by a high CD30 expression. Compared to Doxil®, the CD30-targeted Doxil® showed a significantly higher binding affinity to ALCL cells (5.3% versus 27%, $p = 0.005$) and a lower inhibitory concentration at 50% (IC50) *in-vitro* (32.6 μg/mL versus 12.6 μg/mL, $p = 0.006$). In a SCID mouse xenograft model, CD30-targeted Doxil® inhibited tumor growth more significantly than the unconjugated formulation; specifically, tumors in mice treated with CD30-targeted Doxil® were significantly smaller than those in mice treated with Doxil® (average, 117 mm^3 versus 270 mm^3, $p = 0.001$) at 18 days after the tumors were inoculated. Our findings have provided the proof-of-principle of using CD30-targeted nano-carriers to treat cancers that are characterized by a high level of CD30 expression, such as ALCL.

1. Introduction

In recent years, accumulating evidence has suggested that the use of nano-carriers can be more effective than free drugs in delivering anti-cancer agents [1–5]. The most important feature of nano-carriers that accounts for their superior drug delivery is their size range. Specifically, since intra-tumoral blood vessels are often more leaky as compared to the healthy blood vessels, the unique size range of nano-carriers allows them to diffuse into tumors while they are too large to diffuse into normal tissues [6,7]. Furthermore, since the intra-tumoral lymphatics are often poorly formed, nano-carriers that have diffused into tumors cannot be effectively drained out, again resulting in their preferential accumulation in tumors [6–8]. Liposomal carriers, which were first described in 1966, have been extensively investigated as a tool to improve the efficacy of drug delivery [4]. A number of liposomal formulations have already been approved for clinical use, as exemplified by liposomal doxorubicin (Doxil® or Caelyx®). As compared to free doxorubicin, this liposomal formulation has been shown to be superior in drug delivery and the overall therapeutic efficacy. The liposomal formulation of doxorubicin has been used widely in treating various types of cancer [4,9,10].

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More recent studies have suggested that the efficiency of nano-carriers as a drug delivery tool can be further improved through modification of nanocarrier surface with various moieties designed for active targeting of cancer cells, thereby increasing drug delivery to tumor cells [11–14]. To date, various ligands including antibodies (or their fragments), small organic molecules, carbohydrates, and peptides have been successfully coupled to various forms of nano-carriers [14]. Among these moieties, antibodies have been studied most extensively. Nevertheless, the use of antibody-conjugated nano-carriers to treat cancer remains to be in its infancy; to our knowledge, none of these nanoparticles have been approved for clinical use, and only a small number of these agents are being tested in clinical trials [15,16]. One of the potential limitations associated with the use of antibodies for drug delivery is the absorption of these antibodies by circulating free soluble antigens, leading to the neutralization of these antibodies before reaching to their targets. To overcome this problem, one strategy is to generate antibodies which only recognize the cell membrane-bound epitopes, thereby avoiding the neutralization of these antibodies by soluble antigens present in the circulation [17].

Anaplastic large cell lymphoma (ALCL) is a type of T-cell malignant neoplasm characterized by a strong and uniform expression of CD30, which is a member of the tumor necrosis factor receptor superfamily [18,19]. Importantly, CD30 expression is highly restricted in normal tissues, being found largely in a small subset of activated T-lymphocytes [19,20]. Thus, CD30 may serve as a potential target specific for CD30-expressing malignancies such as ALCL. To further justify that CD30 is an excellent target, it has been previously reported that ligation of CD30 in ALCL cells can directly induce both cell cycle arrest and apoptosis [21,22]. A previous study also has shown that anti-CD30-conjugated drugs have the propensity of being internalized by cells through clathrin-mediated endocytosis [23], a feature associated with a high efficiency of drug delivery [24]. Taken together, CD30 appears to be an excellent candidate for serving as a tumor-seeking molecule for nano-carriers used to treat CD30-positive malignancies. Based on this concept, SGN-30, a chimeric anti-CD30 monoclonal antibody, was developed. Clinical trial studies have found the clinical benefit of SGN-30 in patients with primary cutaneous ALCL; importantly, SGN-30 was well tolerated by patients included in these studies [25,26]. The safety and modest clinical activity of SGN-30 was also reported in patients with systemic ALCL [27]. Brentuximab vedotin (SGN-35), an intravenously administered CD30-specific antibody-drug conjugate, has recently been clinically approved for the therapy of Hodgkin's lymphoma and ALCL [28]. Nevertheless, the combination of anti-CD30 and nanomedicines has not yet been attempted.

In this study, we aimed to test if the conjugation of anti-CD30 monoclonal antibody to Doxil®, a liposomal formulation of doxorubicin, which is a component of the current standard frontline regimen for ALCL [29], can result in higher therapeutic efficacy against these cancer cells. The design of these nano-carriers is based on the benefits associated with the use of nanoparticles and anti-CD30, as described here. One additional experimental consideration in this study is that we employed a unique anti-CD30 antibody that recognizes an epitope found only on CD30 expressed on the cell surface but not circulating soluble CD30 fragments.

2. Materials and methods

2.1. Reagents

Methoxypoly(ethylene glycol) (Mw 2000 Da), covalently linked to distearoyphosphadidylethanoloamine ($mPEG_{2000}$-DSPE) were from ALZA Corporation. 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[succinimidyl (polyethylene glycol)] (DSPE-PEG_{3400}-NHS) was purchased from Nanocs Inc. (USA). 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethyelen glycol)2000] (DSPE-PEG_{2000}-NH_2) was purchased from Avanti Polar Lipids, Inc (USA). Sephadex G-50, Sepharose CL-4B and Cy5.5-monofunctional reactive dye were purchased from GE Healthcare (Baie D'Urfe, PQ). Bio-Rad Protein Assay Reagent was purchased from Bio-Rad Laboratories (Mississaguga, ON). FITC and Cy5.5 dyes were obtained from Invitrogen (Eugene, Oregon, USA).

2.2. Cell lines and tissue culture

Two ALCL cell lines, KARPAS 299 and SUP-M2 which over-express CD30 [30,31] were grown and expanded in RPMI 1640 (Sigma–Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS, Sigma–Aldrich, St, Louis, MO) and 2 mM L-glutamine (Gibco, Grand Island, NY, USA) in 5% CO_2 atmosphere at 37 °C.

2.3. Modification of Doxil® with anti-CD30 monoclonal antibody

Doxil®, a commercially available preparation of doxorubicin in PEGylated liposomes was from Schering Plough (Kirkland QC, Canada). The anti-CD30 monoclonal antibody was purified from a hybridoma cell line (T408) [17]. Anti-CD30 antibody was purified using a rapid antibody purification kit from Cell Biolab Inc (San Diego, CA, USA). Cy5.5 labeled anti-CD30 antibody was prepared by adding 1 mg anti-CD30 antibody in PBS to 1 mg of Cy5.5 monofunctional reactive dye, followed by incubation with stirring at room temperature in the dark for 1 h Cy5.5 labeled anti-CD30 antibodies were purified by a G50 column using PBS (pH7.2) as the mobile phase. The conjugation efficiency of Cy5.5 to anti-CD30 antibodies was determined by a UV/visible spectrophotometer measuring the extinction coefficient at 675 nm, as described by the manufacturer. Stealth immunoliposomes were prepared using a modification of the post-insertion method. First anti-CD30 antibody (unlabeled or labeled with Cy5.5) was conjugated to PEG_{3400}-DSPE by adding 2 mg of DSPE-PEG_{3400}-NHS to anti-CD30 antibody solution (1 mg/mL) in PBS. The mixture was kept stirring for 4 h at room temperature. Then, excess amount of glycine (5 mg) was added to the solution to quench the reaction. The resulted micelle solution was purified by Sepharose CL 4B using PBS buffer (pH7.2) as the mobile phase. Then CD30-targeted and non-targeted mixed micelles were prepared from lipid compositions of Cy5.5 or anti-CD30 antibody or Cy5.5 labeled anti-CD30 antibody conjugated to PEG_{3400}-DSPE and $mPEG_{2000}$-DSPE (1:3, weight ratio). To prepare CD30-targeted Doxil®, CD30-targeted or non-targeted mixed PEG_{3400}-DSPE micelles (~2 mg equivalent lipid in 0.5 mL PBS) were incubated with 1 mL of Doxil® (2 mg doxorubicin) for 4 h at 60 C in a water bath with continuous stirring. Un-conjugated antibody and antibody-conjugated lipid micelles were separated from CD30-targeted Doxil® by chromatography on Sepharose CL-4B columns in PBS (pH7.2). The purified Cy5.5 labeled Doxil® and Cy5.5 labeled CD30-targeted Doxil® formulations were visualized by doxorubicin fluorescence and near infrared fluorescence (NIRF) using appropriate filters with a Kodak image station (Imaging Station IS4000-MM, Kodak). Formulations were placed into a imaging system equipped with band-pass excitation and long-pass emission filters at 630 and 700 nm for NIRF and 465 and 570 for doxorubicin fluorescence imaging (Chroma Technology Corporation, Rockingham, VT). Mean diameter and polydispersity of liposomes were defined by light scattering (3000 HS_A Zetasizer Malvern Zeta-Plus™ zeta potential analyzer, Malvern Instrument Ltd., UK). The anti-CD30 density on the liposomes was determined by protein assay [32]. The concentration of doxorubicin in antibody-conjugated liposomes was determined by measuring its absorbance at 485 nm using FLUOstar Omega microplate reader (BMG-LABTECH Cary, NC, USA).

2.4. In vitro cellular binding

To evaluate binding of CD30-targeted nano-carriers to ALCL cell lines, 1×10^6 of SUP-M2 cells (a CD30 positive ALCL cell line) [31] suspended in media were incubated with either of the following formulations for 30 min at 4 °C: Cy5.5 labeled PEG_{2000}-DSPE micelles (non-targeted) or Cy5.5 labeled CD30-targeted PEG-DSPE micelles at a lipid concentration of 20 ug/mL, Cy5.5 labeled Doxil® (non-targeted) or Cy5.5 labeled CD30-targeted Doxil® at doxorubicin concentration of 2.5 ug/mL. Cells were then washed twice with cold PBS buffer (pH7.2) and the cell-associated fluorescence was quantified by flow cytometry (Becton–Dickinson Instruments, Franklin lakes, NJ). Flow cytometry was performed using a 488 nm argon laser and FL4 band-pass emission (at 695 nm) for the near infrared Cy5.5. Fluorescence histograms and dot plots were generated using Cell Quest software (for figures, histograms were recreated using FCSexpress software).

2.5. Cytotoxicity study

In vitro cytotoxicity was determined by the MTT tetrazolium assay as previously described [33]. SUP-M2 cells were incubated with Doxil® and CD30-targeted Doxil® in media for 48 h. 20 uL of MTT (5 mg/mL) was added to each well and incubated for another 4 h to develop the color. After centrifugation, the media was replaced by 200 uL of DMSO. The absorbance was read by a microplate reader. Data are expressed as concentration of doxorubicin that gives a 50% inhibition of cell growth compared to untreated control cells (IC_{50}).