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Research paper

Pharmacokinetics of intravenously administered stealth liposomal doxorubicin modulated with verapamil in rats

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Abstract

Treatment of cancer through co-administration of anticancer drugs and multidrug resistance (MDR) modulators as a strategy to overcome drug resistance has been extensively explored. However, success has been limited by pharmacokinetic interactions because of non-specific blockade of P-glycoprotein (P-gp) in normal tissues or inability to reach relevant concentrations clinically. We hypothesized that stealth liposomal co-encapsulation of doxorubicin (DOX) with a P-glycoprotein inhibitor, verapamil (DARSLs), may overcome these limitations. Using intravenous (i.v.) administrations, the effects of verapamil (VER) either free (FV) or liposome co-encapsulated with DOX (DARSLs) on the pharmacokinetics and tissue distribution characteristics of DOX either as free (FD) or liposome-encapsulated (LD) were evaluated in normal rats. FV increased ($P<0.05$) the plasma AUC of free DOX (FD). Preparations containing LD had significant prolonged systemic exposure and slow tissue distribution of DOX. LDFV (liposomal DOX with free verapamil) and DARSLs shared similar DOX pharmacokinetics but the latter showed slower DOX distribution in most tissues studied and slower ($P<0.05$) DOX biliary transport. The addition of VER into LD in these two preparations significantly increased the AUC ($P<0.01$) and reduced the clearance ($P<0.01$) of DOX when compared to LD. Specifically, DARSLs reduced initial DOX distribution to the heart ($P<0.05$) corresponding to initial alleviation ($P<0.05$) of bradycardia when compared to other DOX with VER preparations. In conclusion, liposomal co-encapsulation of DOX with VER has promise of significant therapeutic advantages, and should be explored further in therapeutic studies with animal tumor xenograft models.

Keywords: Doxorubicin; Verapamil; Stealth liposomes; Pharmacokinetics; Biodistribution

1. Introduction

P-glycoprotein (P-gp) is a member of the ATP-Binding Cassette superfamily proteins involved in the transport of a wide variety of substrates. It is widely distributed in the intestine, lung, kidney, liver, adrenal gland, blood–brain barrier, and placenta [1,2]. When over-expressed by tumors, P-gp-mediated efflux of drugs with resultant multidrug resistance (MDR) tumor cells is a major obstacle to successful clinical cancer chemotherapy. Therefore, attempts to circumvent this problem through pharmacological inhibition of P-gp during cytotoxic drug administration, for example, using verapamil, cyclosporine, valspodar, GF120918 or LY357739 to enhance intracellular drug accumulation into these MDR cells [3,4] have been studied. However, several possible disadvantages of this approach were anticipated, namely pharmacokinetic interactions arising from a considerable overlap of P-gp and CYP3A4 co-expressed in the excretion and metabolism organs [5–8], toxicity of the MDR modulators at doses required for P-gp inhibition and enhanced accumulation of cytotoxic drugs in normal tissues, causing more toxicity. Increased toxicity has

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been reported in neoplastic patients when doxorubicin (DOX) and verapamil (VER) were co-administered to overcoming multidrug resistance [9,10]. High plasma concentrations (2–6 μM) of VER were required for MDR reversal, much higher than for cardiovascular therapy (0.4–1.2 μM), and as a consequence, had a high potential for cardiotoxicity [11,12]. More specific P-gp modulators with less pharmacological effects on normal tissue have limited success as well.

Stealth liposome-based anticancer chemotherapy offers therapeutic advantages of reduced systemic toxicity combined with selective tumor localization. This is due to liposome longevity in circulation providing sustained release of lower concentrations of cytotoxic drug and to liposome extravasations through the abnormally permeable microvasculature of systemic tumors. It has also been demonstrated that the selective tumor localization of doxorubicin encapsulated in stealth liposomes (DOXIL®) is associated with superior therapeutic activity over free drug activity in various systemic tumor models [13–15]. Given the relative tumor specificity of liposomal encapsulation, less undesired pharmacokinetics interactions with P-gp modulators are expected.

Previous studies have demonstrated that liposomal doxorubicin has less potential for pharmacokinetic drug interaction with P-gp inhibitors like valspodar (PSC833) [16,17]. We hypothesized that co-encapsulation of both cytotoxic and P-gp inhibitor might lead to even greater specificity in drug delivery and intracellular retention of cytotoxic drug in the tumor, thereby enhancing therapeutic index. In this study, we formulated stealth liposomal preparations, one of which is co-encapsulated DOX with VER stealth liposomes (DARSLs or *d*oxorubicin *a*nti*r*esistant *s*tealth *l*iposome*s*) [26], and studied doxorubicin pharmacokinetics and cardiotoxicity of these preparations in rats and compared them with free DOX and VER.

2. Materials and methods

2.1. Materials

Egg phosphatitylcholine (EPC), cholesterol (CHOL), polyethylene glycol–distearoylphosphatitylethanolamine (PEG2000-DSPE) were purchased from NOF (Tokyo, Japan); ammonium sulfate, (±)verapamil hydrochloride were purchased from Sigma (USA); polycarbonate membrane filters were purchased from Millipore Corporation (USA). Doxorubicin (DOX) was obtained from Woo-Shin Med. Co. (Korean); all other chemicals were of the commercially available grade. HPLC system with fluorescence detector was from Waters (USA).

2.2. Liposomes and drug preparation

LD and DARSLs (stealth liposomes co-encapsulated with both DOX and VER) were prepared according to Haran et al. [18]. DARSLs were prepared by incorporation of both DOX with VER into stealth liposomes to form DARSLs according to the following process.

EPC (100 μmol), CHOL, PEG2000-DSPE (50:45:5) were dissolved in chloroform. The chloroform was evaporated to dryness under vacuum with a rotary evaporator (IFQ-85A), and the lipid film was hydrated with 10.0 ml of ammonium sulfate solution (150 mM) by sonication in water bath at 60 °C for 30 min, and a suspension containing blank liposomes was obtained. The liposomes were then extruded five times through each polycarbonate membranes (Nucleopore, USA) of pore sizes 0.6, 0.4, 0.2 μm consecutively to make smaller size of liposomes. The resulting liposomes were dialyzed (MWCO 12,000–14,000; Servapor™, Serva Germany) for 40 h at 4 °C against 100 mM phosphate buffer (pH 7.4).

Only DOX or DOX plus VER were encapsulated in the liposomes using the ammonium sulfate gradient loading procedure (Remote loading). The optimum weight ratio of drug:lipid for LD and DARSLs was 1:5 (DOX:Lipid) and 1:0.11:10 (DOX:VER:Lipid), respectively. The resultant DOX liposomes exhibited a mean particle size ranging between 100 and 130 nm as determined using a Zetasizer (Particle sizing systems, Malvern Instruments Ltd, UK), operating at a wavelength of 633.0 nm. Unencapsulated DOX and VER were removed by centrifugation through sephadex-G50 mini columns; DOX and VER concentrations encapsulated in liposomes were measured by HPLC after disruption of the liposomes with methanol. Encapsulation efficiencies of DOX and VER were more than 90 and 70%, respectively.

Free DOX (FD) formulation was administered with sterile saline. Verapamil hydrochloride (for animal studies) was dissolved in sterile saline to form free VER (FV) formulation and administered via intravenous injection. Liposomal DOX preparations (including LD, LDFV and DARSLs) were diluted with sterile saline to appropriate concentrations as necessary prior to intravenous administration.

2.3. Animal experiments

Animal experimental protocols were designed according to the guidelines of International Guiding Principles for Animals Research (WHO Chronicle, 39 (2): 51–56, 1985; A CIOMS Ethical Code for Animal Experimentation). The protocols were approved by the National University of Singapore Animal Ethics Committee. Male SD rats, weight 200–250 g, were housed under standardized conditions of temperature (22 °C) and exposed to a regular light/dark schedule (7:00–19:00/19:00–7:00). Food and water were provided ad libitum. All animals were kept for 1 week prior to drug administration and were fed a standard rat chow.

The animals were randomly divided into experimental groups (varying from 3 to 15 rats per group) for treatment with saline and formulations of FD, FV, FDFV, LD, LDFV