Current Topics

Challenges of Drug Delivery Systems That Contribute to Cancer Chemotherapy

Development of Liposomal Anticancer Drugs

Kenji Hyodo,*a Eiichi Yamamoto,a Takuya Suzuki,a Hiroshi Kikuchi,b Makoto Asano,c and Hiroshi Ishiharaa

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Liposomes are drug delivery systems that can alter the pharmacokinetic properties of compounds. The adverse effects of anticancer agents are a limiting factor for cancer chemotherapy, therefore, liposomal formulations have the potential to improve the therapeutic efficacy of anticancer agents by enhancing their accumulation in tumors and reducing non-selective distribution to normal tissues, which is known as the enhanced permeability and retention effect. To develop a liposomal anticancer agent as a drug product, its formulation must be designed to ensure its quality until it is administered to patients and to exert maximum potency in clinical use rather than in animal experiments. The chemical stability and physicochemical stability of the ingredients are key factors in the design of liposomal formulations. Drug release rates are critical factors in the therapeutic efficacy of liposomal drug products because the encapsulated drug has no pharmacological activity, and only released drug can exert antitumor/toxic activities. Liposomes should maintain the drug in a stable state in the circulation and then promptly release it after accumulation in the target tissue in order to achieve a sufficient drug concentration. To understand the profile of the formulation and to guarantee the quality of drug product, a reliable analytical method that can determine the released and encapsulated drugs in biological fluids is required. Simple online solid phase extractions of the released and encapsulated drugs using a column-switching HPLC system meet the requirements and this system enables accurate in vitro release testing and in vivo pharmacokinetic evaluation. This review introduces the process of liposomal drug product development from various viewpoints.

Key words liposome; anticancer drug; drug delivery system; enhanced permeability and retention effect; accelerated blood clearance phenomenon; therapeutic efficacy

1. INTRODUCTION

Twelve liposomal drug products have already been launched in the world and many other liposomal drugs are in clinical trials.34 We are developing liposomal Eribulin as a new liposomal drug product and it is currently in the phase I clinical trial stage. Particles such as liposomes and macromolecular drug carriers such as polymers are classified as nanomedicines, a field encompassing nano-scale drug delivery devices that aims at increased sophistication in selectivity and control of drug delivery.23 The liposome platform has been extensively studied as a tool to encapsulate drugs. Addition of a conjugate of polyethylene glycol (PEG) linked to a lipid anchor (distearoyl-phosphatidylethanolamine) to the liposomal formulation was shown to significantly prolong liposome circulation time. PEG-coating contributes to steric stabilization of the vesicles and provides important protection from opsonisation, resulting in delayed hepatic reticuloendothelial system (RES) clearance and greatly extending circulation time.30 Indeed, a hallmark of the long-circulating PEG-modified liposomal drug carriers is their enhanced accumulation in tumors.29 The mechanism underlying this passive targeting effect is the phenomenon known as enhanced permeability and retention (EPR). Initially described by Maeda37 to account for increased deposition of macromolecular drug carriers in tumors, the EPR effect also applies to liposomes and other nanoparticles. The EPR effect9 is related to the increased vascular permeability of tumor vessels characteristic of the tumor neoangiogenic process. Tumor microvessels have, among other abnormalities,9 large fenestrations that enable extravasation of macromolecules and liposomes. Therefore, PEG-modified liposomes of less than 100 nm in diameter accumulate in a tumor based on this EPR effect. These features of liposomal formulation enable improvement of pharmacological potency, reduction of dosing frequency and expansion of indication. This leads to an improvement of the quality of life (QoL) of cancer patients. Here we review the development process for liposomal anticancer drugs, using doxorubicin as a model compound.

2. DESIGNING A FORMULATION TO ACHIEVE OPTIMIZED PROPERTIES AS DRUG PRODUCT

The target product profile, such as drug concentration, lipid composition, lipid concentration and buffer composition, will
be determined by manufacturability, physicochemical stability, chemical stability of active and inactive pharmaceutical ingredients, and convenience in clinical usage. The drug to lipid ratio is a key factor in liposomal formulation because it influences various important aspects of the development process, such as manufacturability, pharmacokinetic profile and costs. In terms of manufacturability, a higher drug to lipid ratio is desirable because sterile filtration is the most challenging step in the manufacture of a liposomal formulation. Liposome solutions with a high lipid concentration can easily result in filter clogging in the sterile filtration step, therefore reduction of the lipid concentration by achieving a higher drug to lipid ratio is desirable. Interior and exterior buffer compositions are determined by ingredient stability and the loading method. Doxorubicin is an amphipathic weak base with a pK_a of 8.22 and logP of 1.4. There are various loading methods applicable to doxorubicin. Among them the ammonium ion gradient method and pH gradient method are the most effective to achieve a high drug to lipid ratio and their acidic pH in the liposome interior is favorable for the chemical stability of doxorubicin. Higher exterior buffer pH is better from the point of view of encapsulation efficiency, however, the exterior buffer pH should be kept below 7.0 during the manufacturing process because doxorubicin is extremely unstable in alkaline pH. The most important factor for the determination of a formulation is to ensure the quality of drug product until administration to the patient.

The pharmacological efficacy of a liposomal formulation is dependent on the rates at which the drugs are released from liposomes, especially in cell cycle dependent anticancer agents, such as vincristine. Doxorubicin, unlike vincristine, is not critically dependent on the exposure time, but there is an appropriate release rate for liposomal doxorubicin to show superior pharmacological activity. Only released drug has pharmacological activity; liposomes should retain encapsulated drug in the blood circulation and promptly release the drug at rates sufficient to achieve an adequate drug concentration after arrival at the target tissue. Release rates can be controlled by the acyl chain length of the lipid, saturation of the acyl chain, and interior buffer composition. To evaluate the relationship between release rates from liposomes and tumor growth rates, we prepared two types of liposomal doxorubicin. A fast release type was prepared by the pH gradient method with citric acid as interior buffer and a slow release type was also prepared by the ammonium gradient method with ammonium sulfate as interior buffer. A clear difference was observed in the plasma concentration of doxorubicin at 24h after intravenous administration to mice (data not shown). These two types of liposomal formulation showed equivalent antitumor activity in a syngeneic subcutaneous B16 melanoma model, but the slow release type showed significantly more potent efficacy than the fast release type in a subcutaneous Meth A sarcoma model (data not shown). The B16 melanoma model is well known as an aggressive rapid growing tumor model, while on the other hand, the tumor growth rate of the Meth A sarcoma model is slower than that of the B16 model. Indeed, the tumor growth rate is only one factor to affect the therapeutic efficacy of a liposomal formulation, however, we must take into consideration the difference in tumor growth rates between animal models and clinical tumors in order to

Fig. 1. Antitumor Activity of Adriacin and DOXIL When Administered at Maximum Tolerated Dose (10mg/kg for Each Drug Product) at Day 0 and Day 7 to SKOV3 Human Ovarian Cancer Xenograft Mice (A) or FaDu Human Head and Neck Cancer Xenograft Mice (B)
design a therapeutically optimized drug release rate.

3. DEVELOPMENT OF ANALYTICAL METHODS TO ENSURE THE QUALITY OF DRUG PRODUCT

Reliable methods to determine the amounts of encapsulated and released drugs in biological fluids are needed for understanding the pharmacological activity of liposomal drugs and to guarantee the quality of drug product. The U.S. Food and Drug Administration released “Draft Guidance on Doxorubicin Hydrochloride” in 2010 and the European Medicines Agency also released a draft version entitled “Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product” for the development of generic liposomal drug products. These draft guidances were released to discuss approaches that satisfy the requirements of the applicable statutes and regulations and not with the intention to define specific analytical methods to evaluate bioequivalence with original drugs. However, physicochemical parameters with which to guarantee the equivalence between original and generic drug products are important factors for characterizing the properties of a new liposomal drug product. Investigation of the requirements and analytical methods described in the guidances will provide useful insight into not only generic product development but also the creation of new liposomal drug products. Both draft guidances recommend analyzing encapsulated and released drugs separately to estimate the pharmacokinetic properties and release profiles of the drug product. Analytical methods for the measurement of released and encapsulated drugs in plasma samples have previously been reported. However, these methods need complex sample preparation procedures, such as ultracentrifugation, solid phase extraction (SPE), gel filtration or ultrafiltration before analysis. These separation methods have a potential risk of sample deterioration: adsorption to ultrafiltration devices, high sample dilution during gel chromatography, and drug release from the liposomes during reversed phase SPE. To circumvent these difficulties, we developed simple online SPE of released and encapsulated drugs in the plasma using a column-switching HPLC system.

Fig. 2. ABC Phenomenon of PEG-Modified Liposomes
Typical image of ABC phenomenon (A) and suggested mechanism (B).
and a methylcellulose-immobilized octadecysilylated silica SPE column, which is a restricted access media column capable of direct plasma injection. This direct plasma injection approach using the proposed SPE–SPE–HPLC system made it possible to simultaneously measure the encapsulated and released drugs in plasma. The methodology enabled us to determine the in vivo properties of liposomes and contribute to the efficient creation of liposomal drug product.

4. PHARMACOLOGY AND SAFETY STUDIES TO INITIATE CLINICAL TRIALS

Pharmacology studies using various types of human tumor xenograft models are performed after the candidate formulation for clinical trials is established. Each model has a different drug-sensitivity and tumor microenvironment. These studies are intended to characterize the pharmacological activity of the formulation and to explore the optimal dosing schedule and suitable tumor type to support the clinical trials. Taking DOXIL, a liposomal formulation of doxorubicin launched by Janssen Pharmaceuticals K.K., as a model liposomal drug product, we conducted some pharmacology studies in various human tumor xenograft models. To evaluate the benefits of liposomal formulation, Adriacin, an unencapsulated formulation of doxorubicin launched by Kyowa Hakko Kirin Co., Ltd., was used as a control. Each xenograft model showed a different response when DOXIL or Adriacin was administered at the maximum tolerated dose (10 mg/kg for each drug product). Figure 1 shows a typical example of the difference in antitumor activity of DOXIL and Adriacin. DOXIL clearly showed more potent antitumor activity in the SKOV3 xenograft model compared to Adriacin, although DOXIL showed antitumor activity similar to that of Adriacin in the FaDu xenograft model. These studies suggest that DOXIL will show potent antitumor activity in patients who have a background similar to that of the SKOV3 model. The superiority of liposomal formulations to unencapsulated formulations with respect to antitumor activity depends on the models investigated and the physicochemical properties of the liposomal formulation. Elucidation of the underlying mechanisms in order to verify the superiority of liposomal formulations versus unencapsulated formulations will lead to the efficient development of liposomal anticancer drugs.

To initiate clinical trials, safety pharmacology studies in compliance with Good Laboratory Practice are necessary to identify undesirable pharmacodynamic properties of the drug product and to investigate the mechanism of the adverse pharmacodynamic effects. In these studies, dose-escalation, animal scale-up with rodents and non-rods, and repeated administration are required. Recently, the phenomenon of accelerated blood clearance (ABC) of repeatedly administered PEG-modified liposomes has been reported (Fig. 2A). This phenomenon is caused by the anti-PEG immunoglobulin M (IgM) produced in response to the first administration of PEG-modified liposomes (Fig. 2B), however, it was also reported that PEG-modified liposomes encapsulating doxorubicin do not elicit this phenomenon because the released doxorubicin damage the immune cells, such as IgM-producing B cells. Clarification of the condition that elicits ABC is very important to evaluate the safety pharmacological properties of PEG-modified liposomes encapsulating anticancer agents accurately. Some pharmacokinetic studies were conducted using DOXIL as model drug product and we have reported that DOXIL administered at a therapeutic dose (20 mg/m²) did not cause the ABC phenomenon, but DOXIL administered at lower doses (<2 mg/m²) caused anti-PEG IgM production and thereby a rapid clearance of the second and third administration of DOXIL in Beagle dogs. Our results indicate that the ABC phenomenon is not an issue with DOXIL in therapeutic dose range and also may not be for the other PEG-modified liposomes encapsulating cytotoxic drug at therapeutic dose ranges. These results suggest that the pre-clinical study of PEG-modified liposomes containing anti-cancer agents must be carefully designed and performed with monitoring of the anti-PEG IgM level and the liposomal drug concentration in the blood.

5. CONCLUDING REMARKS

Recently, the discovery of new molecular entities by the pharmaceutical industry has become increasingly difficult. Under such circumstances, drug delivery systems (DDS) have been attracting increasing attention from the pharmaceutical industry because they can provide new values to existing active compounds. Liposomal drug delivery is the most reliable DDS to create new drug product. There are currently twelve liposomal drug products on the market, which means liposome technology is well-established and well-accepted by clinicians. We should see many more liposomal drug products in the future and they will contribute to the improvement of patient QOL.

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