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GUIDANCE FOR INDUSTRY[1]

Liposome Drug Products

Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation

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If you plan to submit comments on this draft guidance, to expedite FDA review of your comments, please:

- *Clearly explain each issue and/or concern and, when appropriate, include a proposed revision and the rationale or justification for the proposed change.*
- *Identify specific comments by line number or numbers; use the pdf version of the document, whenever possible.*
- *If possible, send an electronic copy (Word or WordPerfect) of the comments you have submitted to the docket by e-mail to* coryj@cder.fda.gov.

I. INTRODUCTION

This guidance provides recommendations to applicants on the chemistry, manufacturing, and controls (CMC); human pharmacokinetics and bioavailability; and labeling documentation for liposome drug products submitted in new drug applications (NDAs).[2] The recommendations in this guidance focus on the unique technical aspects of liposome drug products. Applicants should

[1] This guidance was prepared by the Liposome Working Group of the Complex Drug Substances Coordinating Committee (CDSCC) in the Center for Drug Evaluation and Research (CDER) at the FDA.

[2] A liposomal formulation of an active moiety that has already been approved or marketed in the United States is not classified as a new molecular entity (Type 1 NDA). When submitted in an NDA, the drug is classified as a Type 3 NDA unless it is a new ester, new salt, or other noncovalent derivative of the approved drug substance. In that case, the NDA would be classified as a Type 2,3.

refer to the forthcoming drug product guidance[3] for other recommendations on the CMC documentation that should be submitted in original NDAs. Applicants can contact the appropriate review division if they have questions on demonstrating bioequivalence and sameness of liposome drug products. The recommendations in this guidance should be considered, to the extent applicable, when a sponsor is submitting an investigational new drug application (IND).

Liposome drug products are defined as drug products containing drug substances (active pharmaceutical ingredients) encapsulated in liposomes. A liposome is a microvesicle composed of a bilayer of lipid amphipathic molecules enclosing an aqueous compartment. Liposome drug products are formed when a liposome is used to encapsulate a drug substance within the lipid bilayer or in the interior aqueous space of the liposome. A drug substance in a liposome formulation is intended to exhibit a different pharmacokinetic and/or tissue distribution (PK/TD) profile from the same drug substance (or active moiety) in a nonliposomal formulation given by the same route of administration. The complete characterization of the PK/TD profile of a new liposome drug product is essential to establish the safe and effective dosing regimen of the product.

The guidance does not provide recommendations on:

- clinical efficacy and safety studies
- nonclinical pharmacology and/or toxicology studies
- bioequivalence studies or those to document sameness
- liposomal formulations of vaccine adjuvants or biologics
- drug-lipid complexes[4]

II. CHEMISTRY, MANUFACTURING, AND CONTROLS

The recommendations provided on CMC documentation focus on the information specific to liposome drug products that should be submitted to CDER. An applicant should consult all relevant regulations and guidances for information on the type of documentation that should be submitted for drug substances and other aspects of documenting the identity, strength, quality, purity, and potency of the drug product.

A. Description and Composition

[3] This guidance is under development and, when finalized, will replace the guidance for industry *Submitting Documentation for the Manufacturing of and Controls for Drug Products*. CDER guidance documents can be found on the Internet at http://www.fda.gov/cder/guidance/index.htm. We update guidances periodically. To make sure you have the most recent version of a guidance, check the CDER guidance Web site.

[4] Drug-lipid complexes are formed by mixing a drug with lipids in such a way that true liposomes are not created. The CMC, pharmacokinetics, and bioavailability recommendations for drug-lipid complexes and liposomes can be similar. Applicants intending to submit an NDA for a drug-lipid complex can consult the appropriate review division in CDER for additional guidance if necessary.

The components of liposome drug products are the drug substance, the lipids, and other inactive ingredients. The quantity of lipid in the formulation should be expressed as the molar ratio and percentage by weight of the lipid to the drug substance as well as on the milligram (mg) per milliliter (mL) and per vial basis. The pharmacological and toxicological properties and the quality of these drug products can vary significantly with changes in the formulation, including the lipid composition. Therefore, any ranges in the formulation components should be specified and be as narrow as feasible. The drug product composition and any specified ranges for components should be justified.

B. Physicochemical Properties

The physicochemical properties of the liposome drug product are critical to ensuring drug product quality. Therefore, a detailed evaluation of these properties should be provided. Rigorous characterization of the physicochemical properties can also be beneficial in evaluating subsequent changes in manufacturing (see section II.G. on Changes in Manufacturing). The physicochemical characterization tests, which are critical to ensuring product quality of each batch of liposome drug product, should be identified. However, all the characterization tests need not be included in the specifications. Properties specific to liposome drug products that may be useful to assess include:

- morphology of the liposome, including lamellarity determination, if applicable
- net charge
- volume of entrapment in liposomal vesicles
- particle size (mean and distribution profile)
- phase transition temperature
- spectroscopic data, as applicable
- in vitro release of the drug substance from the liposome drug product
- osmotic properties
- light scattering index

C. Description of Manufacturing Process and Process Controls

Liposome drug products are sensitive to changes in the manufacturing conditions, including changes in scale. This should be considered during the development process, and critical manufacturing parameters (e.g., scale, shear force, temperature) should be identified and evaluated. If there are changes in critical manufacturing parameters, complete characterization of the liposome drug product is recommended and in vivo studies may be warranted (see section II.G. on Changes in Manufacturing).

The chemical and physical complexity of liposome drug products can provide unique challenges to the sterilizing filtration process. For example, constituents of the liposome may block adsorptive interactions of organisms with the filter matrix, effectively allowing organisms to pass through the sterilizing filter. Therefore, product-specific validation studies should demonstrate the microbial retentivity of the intended sterilizing filters.

D. Control of Excipients: Lipid Components

The quality and purity of the lipid components can affect the quality of the liposome drug product. Information concerning the CMC of the lipid components should be provided at the same level of detail expected for a drug substance. For further information, refer to the guidance for industry *Submitting Supporting Documentation in Drug Applications for the Manufacture of Drug Substances* (the drug substance guidance). This information can be provided in a Type IV Drug Master File (DMF). (See guidance for industry *Guideline for Drug Master Files*).

In addition to the information recommended by the drug substance guidance, recommendations specific to lipid components are provided below.

1. Description and Characterization

If the lipid is a well-defined synthetic or semisynthetic lipid, such as dimyristoylphosphatidylcholine (DMPC), a structure proof based upon standard spectroscopic techniques is usually sufficient. In the case of natural lipid mixtures (e.g., egg lecithin), the lipid composition (i.e., percentage of each lipid) and the fatty acid composition (i.e., the percentage of each fatty acid) should be provided.

2. Manufacture

For synthetic lipids, the source (e.g., manufacturer) and specifications for any starting materials should be provided. For natural lipid mixtures and natural-sourced materials that start the synthetic segment of a semisynthetic process, the biological source (e.g., eggs), country of origin of the source material, supplier, and specifications should be provided.

A complete description of the synthetic process, extraction, and purification procedures should be provided, as applicable. Specifications should be provided for starting materials, raw materials, solvents, and reagents. The controls for critical steps and intermediates should be provided. Chromatographic purification procedures should be described, including the collection of desired fractions, and a sample chromatogram should be provided. For synthetic and semisynthetic lipids, the manufacturing controls that ensure positional specificity of acyl side chains should be provided, if applicable. (See the drug substance guidance for additional information on the manufacturing information that should be provided.)

Procedures to ensure the removal of animal proteins and viruses should be described, where applicable. Bovine-derived materials should not be imported from countries that are defined as bovine spongiform encephalopathy countries by the U.S. Department of Agriculture (see 9 CFR 94.11).

3. Specifications

A full description of the tests, procedures, and acceptance criteria for the lipid components should be provided. Reference standards should be established and their preparation, qualifications, and storage conditions should be described. In general, the analytical procedures should be validated and the specifications should include a stability-indicating assay. Impurities, including possible synthetic by-products, should be evaluated. The level that would warrant identification and qualification will be determined on a case-by-case basis.

For synthetic lipids such as DMPC and semisynthetic lipids, the assay and impurity tests can be done by comparison with the reference standard (e.g., thin-layer chromatography (TLC)) when the analytical procedure can distinguish the desired lipids from possible impurities. If the analytical procedure cannot distinguish the desired lipids from impurities, then assays capable of confirming the fatty acid composition and positional specificity should be used.

For natural lipid mixtures such as egg lecithin, the specifications should be sufficient to ensure that the lipid can perform adequately in the liposome drug product and conform to impurity limits. Based on the nature of lipid or lipid mixtures, the lipid composition (e.g., percentage of each lipid and fatty acid, positional specificity of acyl side chains, degree of fatty acid unsaturation) should be specified in some circumstances. For instance, if the degree of unsaturation of the fatty acid side chains is too high, stable liposomes might not be formed. If the data indicate that this is a critical factor, acceptance criteria for the degree of fatty acid unsaturation should be included in the specifications. Other examples of parameters that can be critical to the performance of the lipid are the amount of phosphatidylglycerol or phosphatidylserine in a *lecithin* preparation.

4. Stability

Lipids used to manufacture liposomes should undergo stability studies to establish the storage conditions and retest date or shelf life. Stress testing (i.e., high temperature, light, pH, and oxygen), should be performed to determine the degradation profile. The container and closure system for storage and shipment of the lipids should be described, and relevant stability data should be provided.

E. Control of Drug Product: Specifications

For recommendations on specifications, applicants should consult the International Conference on Harmonisation (ICH) guidance for industry *Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances*, where appropriate. Additional testing specific to liposome drug products is recommended over that which is typical of the nonliposomal dosage form. Additional tests may include, for example:

- physicochemical parameters of the liposome determined to be critical to product quality for each batch (see section II. B. on Physicochemical Characterization)

- assay for encapsulated and unencapsulated (i.e., free) drug substance
- degradation products related to the lipids
- assay of lipid components
- in vitro test for release of drug substance from the liposome (see section III.D on In Vitro Stability)

F. Stability

The concepts in the CDER guidance for industry *Submitting Documentation for the Stability of Human Drugs and Biologics* [5] and the ICH guidance *Q1AR Stability Testing of New Drug Substances and Products* apply to the design of stability studies for liposome drug products. In general, stability studies should address both physical and chemical stability of the liposome drug product, including the liposome itself. Stability testing of unloaded liposomes (i.e., liposomes to be combined with a drug substance before use) should also be performed. Stress testing of liposome drug products and unloaded liposomes may be warranted to demonstrate possible degradation or other reaction processes unique to the liposomes.

The physical stability of liposome drug products is a function of the integrity and the size distribution of the lipid vesicles. Liposomes are susceptible to fusion, aggregation, and leakage of the encapsulated drug substance during storage. For instance, small unilamellar vesicles are more susceptible to size changes than are multilamellar vesicles. Also, the type of lipids in the bilayer or the encapsulated drug substance may affect fusion of the liposomes or leakage of drug substance from the liposome. Therefore, tests for physical parameters should be developed to assess the integrity and size of the liposomes.

Liposome drug products should be evaluated for stability of the encapsulated drug substance as well as stability of the lipids that compose the liposomal bilayer. Lipids with unsaturated fatty acids are subject to oxidative degradation, while both saturated and unsaturated lipids are subject to hydrolysis to form lysolipids and free fatty acids. Therefore, tests should be developed to evaluate the chemical stability of the lipids in the liposome drug product.

G. Changes in Manufacturing

Manufacturing changes outside of the variations allowed in the approved application must be reported to FDA, as described in section 506A of the Federal Food, Drug, and Cosmetic Act (the Act) (21 U.S.C. 356a). CDER's guidance for industry *Changes to an Approved NDA or ANDA* should be consulted for recommended reporting mechanisms. All changes should be performed in accordance with written change control procedures established by the manufacturer.

[5] The 1987 stability guidance will be superseded by FDA's draft guidance for industry *Stability Testing of Drug Substances and Drug Products* (June 1998) once it is issued in final form.

Because liposome drug products are a relatively new dosage form, it is not possible to provide recommendations on the type of information that should be generated to demonstrate that the change has not adversely affected the quality of the drug product. The extent of the information and documentation to be developed and submitted to support a change should depend on the types of manufacturing changes and the stage of manufacturing at which the changes occur. In general, the information should include testing routinely used for batch release of liposome drug products (see section II.E on Specifications) and depending on the type of change, additional tests specifically directed at evaluating the effect of the change on the liposome drug product (see section II.B. on Physicochemical Properties). In vivo studies may be warranted to demonstrate that the changed product is equivalent to the original product with respect to safety and efficacy.

Before distributing the product made with a change, applicants must assess the effect of each manufacturing change (including site changes, changes to the lipid composition and lipid component specifications) on the identity, strength, quality, purity, and potency of the liposome drug product, as these factors relate to safety and efficacy (section 506A(b) of the Act). The liposome drug product resulting from these changes (i.e., postchange product) should usually be compared to the liposome drug product manufactured as approved in the application (i.e., prechange product). Comparison testing of prechange and postchange drug products should be performed to initially characterize the changed product but is not necessary for routine testing after the change is implemented. An applicant can contact the appropriate review division if it has questions on the type of information to generate or the appropriate reporting mechanism for a postapproval change.

III. HUMAN PHARMACOKINETICS AND BIOAVAILABILITY

When an NDA is submitted for a liposome drug product, the requirements to provide human pharmacokinetics and bioavailability data apply (see 21 CFR 314.50, 320.21, and 320.29). This guidance does not provide information on clinical pharmacology and/or clinical efficacy and safety studies that would be submitted in an NDA. Applicants should consult relevant guidance documents for recommendations on the information to be provided for these topics, or they can consult the appropriate CDER review division.

A. Bioanalytical Methods

Validated bioanalytical methods should be used when evaluating the pharmacokinetics and bioavailablity of a drug substance.[6] For liposome drug products the bioanalytical method should also be capable of measuring encapsulated and unencapsulated drug substance. If a method that distinguishes between encapsulated and unencapsulated drug substance cannot be developed, a justification as to why it is not feasible to develop such a method should be provided.

[6] While the term *drug substance* is used throughout section III, it is recognized that the drug substance may exist as the active moiety in vivo.

Additional information on validation of methods can be found in CDER's guidance for industry *Bioanalytical Method Validation.*

B. In Vivo Integrity (Stability) Considerations

In addition to the general stability considerations of the drug substance in a biological fluid, the stability of the liposome in vivo should be considered.

If the bioanalytical method can distinguish between encapsulated and unencapsulated drug substance, the in vivo stability of the liposome should be determined. A single-dose study is recommended to assess the in vivo stability of the liposome. The concentration-time profile should be evaluated at multiple time points over an adequate period of time. The concentration of encapsulated and unencapsulated drug substance should be determined at each sampling time point.

The liposome is considered stable in vivo if, over the time course of the single-dose study, the:

- drug substance, when in circulation, remains substantially in the encapsulated form
- ratio of unencapsulated to encapsulated drug substance remains constant

When the liposome is stable in vivo, the total drug substance concentration can be measured to determine the pharmacokinetics and bioavailability. However, for an unstable liposome drug product, the concentration of both encapsulated and unencapsulated drug substance should be measured.

If an applicant uses a bioanalytical method that does not distinguish encapsulated and unencapsulated drug substance, this method should be justified (see section III.A on Bioanalytical Methods). When the applicant justifies the use of such a method, the total drug substance concentration can be measured to determine pharmacokinetics and bioavailability.

C. Protein Binding

The stability of liposomes in vivo can be affected by interactions with lipoproteins and other proteins in the blood. Such interactions can have safety implications if dose dumping occurs as a result of premature release of the drug substance from the liposomes. Interactions of liposomes with serum proteins and lipoproteins can be dependent on the type of lipids used in formulating the liposomes. The protein (including lipoprotein) binding of the drug substance and liposome drug product should be determined over the expected therapeutic concentration range. The major binding proteins should be identified.

D. In Vitro Stability

A validated in vitro test method should be established that uses an appropriate simulated physiological medium and/or human plasma and acceptance criteria for the in vitro release

of the drug substance from the liposome. An in vitro test that measures the release of the drug substance from the liposome can be important for assessing the (1) quality of a liposome drug product, (2) adequacy of the process controls, (3) release characteristics of the product over time, and (4) the effect of CMC changes (e.g., minor manufacturing process changes or change in site of manufacture). As experience is gained in the manufacturing of a liposome drug product, an in vitro test, rather than an in vivo test, may be useful in characterizing the liposome drug product when manufacturing changes are made.

E. Pharmacokinetics and Bioavailability

To adequately characterize the pharmacokinetics and bioavailability of a drug substance after administration of liposome drug product, the following studies should be performed:

1. *Mass Balance Study*

The Agency recommends a comparative mass-balance study be performed to define and assess the differences in systemic exposure and pharmacokinetic measures or parameters between liposome and nonliposome drug products when (1) the two products have the same active moiety, (2) the two products are given by the same route of administration, and (3) one of the products is already approved for marketing. The disposition and pathways of elimination (including metabolism and excretion) and several important pharmacokinetic measures (Cmax, AUC) and parameters (clearance, volume, half-life) of a liposomal formulation administered intravenously can be different from that of a nonliposomal formulation given by the same route of administration. Although no examples currently exist, absorption could also be altered for liposome drug product when given via non-intravenous routes. For these reasons, if satisfactory[7] mass balance information is already available for the approved drug product, a limited mass balance study can be undertaken for the proposed drug product. In such a study, the quantity of the drug substance excreted via the major route should be compared in sufficient subjects by giving the liposomal and the nonliposomal formulations, using a crossover or a parallel study design.

Comparison of the absorption, distribution, metabolism, and excretion (ADME) of the liposome and nonliposome drug product form should be made, using a crossover or noncrossover study design that employs an appropriate number of subjects. Depending on the drug substance under investigation, the dose of the liposome and nonliposome drug product may be different. The mass balance study should be based on drug substance tagged with a radioactive label (e.g., ^{14}C, ^{3}H) before its incorporation into liposomes to allow for sensitive monitoring of

[7] Rarely, historical pharmacokinetic data for comparative purposes can be considered on a case-by-case basis in lieu of formal comparative mass balance and/or pharmacokinetic study, taking into account the following factors: (1) when and how the historical data was obtained, (2) similarities of study populations (e.g., disease condition), (3) analytical procedures, and (4) data analysis. The appropriate CDER review division should be consulted to determine whether historical data can be relied upon.

radioactive label after administration. Blood (plasma or serum as appropriate), urine, and fecal samples should be collected and assayed for radioactive label. Other routes of elimination should be monitored as appropriate. Both parent drug substances and any metabolites present should be quantitated. If feasible, mass balance studies can use nonlabeled drug moieties and ingredients. However, CDER recommends that a applicant contact the appropriate review division before conducting studies using nonlabeled drug substance.

2 Pharmacokinetic Studies

When given by the same route of administration, the pharmacokinetics of a drug substance in a liposomal formulation are expected to be different from the same drug substance in a nonliposomal formulation. For this reason, the pharmacokinetic studies should include a study to compare the ADME of a liposome and nonliposome drug product when (1) the two products have the same active moiety, (2) the two products are given by the same route of administration, and (3) one of the products is already approved for marketing. This information can be useful in establishing dosing regimens and in developing dose-concentration-response relationships. The detailed design of the study should be based on the anticipated dosing regimen in the intended patient population. These measures or parameters should include area under the plasma concentration versus time curve, peak plasma concentration, time to peak plasma concentration, elimination half-life, volume of distribution, total clearance, renal clearance, and accumulation, as appropriate. (See section III.B on In Vivo Integrity Considerations for recommendations on whether the pharmacokinetic measures or parameters should be based on total drug substance or both encapsulated and unencapsulated drug substance.) Major metabolites associated with the therapeutic or toxic effects of the drug substance should be determined. The following pharmacokinetic studies should be conducted:

- a single-dose pharmacokinetic study; this should be a comparative study between the liposome and nonliposome drug product, when appropriate (see above)
- a multiple-dose study evaluating the pharmacokinetics of the drug substance after administration of the liposome drug product
- a dose-proportionality study over the expected therapeutic dose range after administration of the liposome drug product

A population-pharmacokinetics approach can be used where appropriate. See CDER's guidance for industry *Population Pharmacokinetics*.

3. Additional Pharmacokinetic Studies

The following pharmacokinetic studies should be considered:

a. Food-Effect Studies

Food intake can affect the lipid composition of plasma, which may affect the disposition of liposome drug products. The applicant should consult with the Agency if there are questions regarding the conduct and design of food studies.

b. Drug Interactions and/or Special Populations

Depending on the results of the mass balance and the pharmacokinetic studies, investigation of drug-drug interactions and/or pharmacokinetics in special populations may be warranted. The applicant should consult with the Agency if there are questions regarding the conduct and design of these studies.

c. Exposure-Response Studies

Exposure-response studies should be provided when available.

IV. LABELING

Information on labeling requirements can be found in sections 502(e)(3) and 508(a) of the Act (21 U.S.C. 352(e)(3) and 358(a)) and in parts 201 and 299 (21 CFR parts 201 and 299). Guidance specific to liposome drug products is provided below

A. Product Name

The product name should include the established name, dosage form, terminology to describe that it is a liposome drug product, and, if desired, a proprietary (i.e., brand) name. The descriptive terminology should include the term *liposome* and, when appropriate, such terms as *Type A, Type B, and Type C,* to distinguish one liposome product from other liposomal formulations of the same drug substance that are not therapeutically equivalent. For example:

BrandX (Acetaminophen) Liposome-Type A For Injection

B. Cautionary Notes and Warnings

Liposome encapsulation can substantially affect a drug product's functional properties relative to those of the unencapsulated or nonlipid-associated drug substance. In addition, different liposome products with a common drug substance can vary from one another in the chemical composition and physical form of the lipid component. Such differences may affect the functional properties of these drug products. CDER recommends that when warranted:

- A cautionary note should be included in the description section of the labeling

regarding the fact that liposome drug products may behave differently from nonliposome drug products.

- A warning should be included in the labeling that the liposome drug product is not equivalent to or cannot be substituted for other drug products containing the same drug substance.

C. Dosage and Administration

Under § 201.57(j), reconstitution instructions with supporting data are required for lyophilized liposome drug products (21 CFR 201.57(j)). This information should be provided for both unloaded lyophilized liposomes that are reconstituted with a drug substance-containing solution at the time of use, as well as products in which the drug substance is loaded into the liposome by the manufacturer and then lyophilized. Other issues that should be addressed, as warranted, include storage conditions for the reconstituted drug, robustness of the liposome drug product under varied reconstitution conditions (e.g., degree of shaking), and appropriateness of using in-line filters.

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平成22年度　分担研究報告書

PEGリポソーム製剤の物性評価技術に関する検討

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（研究要旨）
ポリエチレングリコール（PEG）修飾リポソームの血中動態や体内分布に影響する表面物性として，表面電位測定によるリポソーム表面固定水和層の厚さの評価を行った．市販されているドキソルビシン製剤（Doxil）と同等の脂質組成を用い，PEG脂質としてはリン脂質誘導体あるいはジアシルグリセロール誘導体を用いてリポソームを調製した．PEG修飾によってリポソーム粒子径に変化は見られなかったが，その表面ゼータ電位の値はPEG修飾率の増加に伴って小さくなり，PEG鎖による固定水和層の形成が示唆された．そこで，拡散電気二重層の厚さと電解質濃度との関係を表すグイ-チャップマンの理論を適用してPEG鎖による表面固定水和層の厚さを求めたところ，表面PEG脂質濃度に依存した固定水和層の厚さの増大が確認された．以上の結果から，PEG修飾リポソームの表面PEG層の評価方法として，ゼータ電位測定の有用性が示された．

A. 研究目的

PEG修飾リポソームは，リポソーム製剤の血中滞留性を向上させる有効な手法として開発され，ドキソルビシン製剤であるDoxilを初めとしていくつかの製剤が既に実用化されている．PEG修飾リポソームの高い血中滞留性は，リポソーム表面のPEG鎖が形成する水和層やPEG鎖自身の立体障害により，オプソニンなどの血清タンパク質の結合や単核食細胞系などとの相互作用，血漿中でのリポソーム粒子間での凝集などが抑制されるためと考えられている[1-3]．したがって，PEG修飾リポソームのPEG水和層の物理化学的特性は，リポソーム粒子の体内動態ひいては生物学的利用能を決定する重要な因子であるが，その科学的・技術的評価方法は未だ確立されていないのが現状である．そこで本研究では，PEG鎖によるリポソーム表面固定水和層の厚さ（Fixed Aqueous Layer Thickness: FALT）が，リポソーム表面電位（ゼータ電位）測定によって見積もることができることに着目し[4]，拡散電気二重層の厚さと電解質濃度との関係を表すグイ-チャップマンの理論を適用して，PEG修飾リポソームのPEG層の厚さの評価を行った．

B. 研究方法

1. 試薬

リポソーム構成脂質としては，distearoylphosphatidylcholine（DSPC），distearoylphosphatidylglycerol（DSPG）は

日本油脂(株)から，cholesterol は Sigma-Aldrich 社から購入したものを用いた．PEG 脂質としては，分子量 2000 の PEG 鎖が付加したリン脂質誘導体（DSPE-PEG2000）あるいはジアシルグリセロール誘導体（DSG-PEG2000）を，日本油脂(株)から購入して用いた（図 1,2）．

2. リポソームの調製

脂質をメタノール：クロロホルム（1：2 v/v）に溶解後，ロータリーエバポレーターを用いて薄膜を形成させ，一晩減圧乾燥により溶媒を除去した．薄膜に 10mM Tris-HCl 緩衝液（pH 7.4）を加え，約 60℃でボルテックスにより脂質を分散させた．これを 60℃付近に保ったまま，Mini-Extruder（Avanti Polar Lipid 社）を用いて 200nm のポリカーボネートフィルターを十数回通過させてリポソームを調製した．リン脂質の定量は，Bartlett 法[5]により行った．

3. リポソームの粒子径・ゼータ電位の測定

調製したリポソームの粒子径とゼータ電位は，NICOMP 社製 NICOMP 380 ZLS を用いて測定した．測定には光路長 1cm のプラスチックセルを用い，脂質濃度は 0.5mM で行った．

C. 研究結果

1. PEG 修飾リポソームの粒子径とゼータ電位

図 3 に調製したリポソームの粒子径測定結果を示す．未修飾ならびに PEG 修飾リポソームのいずれも平均粒子径が 200nm 程度であり，粒子径分布もよく似ていた．したがって，PEG リン脂質修飾によるリポソーム粒子径の変化は見られないことが確認された．

これに対し，リポソーム表面電位の指標となるゼータ電位の絶対値は，PEG 脂質の表面濃度に依存して小さくなり（図 4），PEG 鎖による固定水和層の形成が示唆された．

2. グイ-チャップマン理論によるリポソーム表面 PEG 鎖の固定水和層の厚さの算出

リポソームのゼータ電位の絶対値は，共存する塩濃度（イオン強度）の増大によって減少する（図 5）．これは，グイ-チャップマン理論による表面電位 ψ_x と粒子表面からの距離 x との関係式

$$\psi_x \approx \psi_0 \exp(-\kappa x) \qquad \cdots (1)$$

から説明される[6]．ここで，ψ_0 は $x=0$ のときの電位を表し，κ はデバイパラメータである．κ はイオン強度 I と

$$\kappa = \sqrt{\frac{2000 N_A e^2 I}{\varepsilon_r \varepsilon_0 kT}} \qquad \cdots (2)$$

の関係にあり，NaCl のような 1：1 電解質の場合，25℃では

$$\kappa = \sqrt{[\mathrm{NaCl}]} / 0.304 \qquad \cdots (3)$$

となる．したがって，NaCl 濃度を変えてゼータ電位 $\zeta(\approx\psi_x)$ を測定することで

$$\ln \zeta = \ln \psi_0 - \kappa x \qquad \cdots (4)$$

の関係から，粒子表面からすべり面までの距離 x，すなわち固定水和層の厚さ（FALT）を求めることができる．

図 6 は，未修飾及び 5.8 mol% の DSPE-PEG2000 あるいは DSG-PEG2000 で修飾したリポソームのゼータ電位とデバイパラメータ κ との関係を表すが，(4)式に従った良好な直線関係がみられている．この直線の傾きから FALT を求め，表面 PEG 脂質濃度との関係を表したのが図 7 であるが，表面 PEG 脂質濃度に依存して PEG 鎖が形成する固定水和層の厚さが増大している様子を示してい

る.

D. 考察

今回，PEG 修飾リポソームのゼータ電位測定が，リポソーム表面に形成される PEG 鎖水和層の厚さの評価として有用である[4]ことが確認できた．しかしながら，本法はあくまでも間接的な方法であり，PEG 修飾リポソームの表面膜水和状態や血漿タンパク質との相互作用の変化などを直接測定し，FALT との相関を検討する必要があろう．また，リポソームの PEG 修飾法として近年多用されている post insertion 法[7]への適用も今後の課題と思われる．

E. 結論

PEG 修飾リポソームのゼータ電位測定結果に，拡散電気二重層の厚さと電解質濃度との関係を表すグイ-チャップマンの理論を適用して，PEG 鎖による表面固定水和層の厚さの評価を行った．その結果，表面 PEG 脂質濃度に依存した固定水和層の厚さの増大が確認され，PEG 修飾リポソームの表面 PEG 層の評価方法としてゼータ電位測定の有用性が示された．

F. 参考文献

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G. 研究発表

なし

H. 知的財産権の出願・登録状況

なし

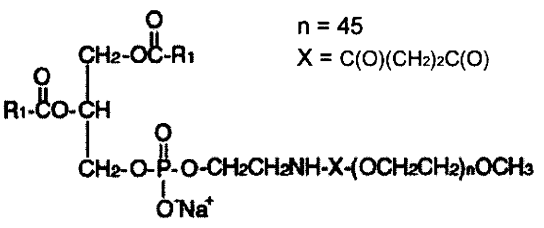

図 1 DSPE-PEG2000 の構造式

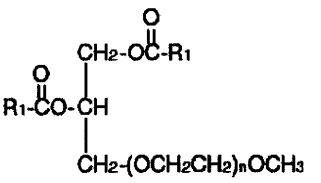

図 2 DSG-PEG2000 の構造式

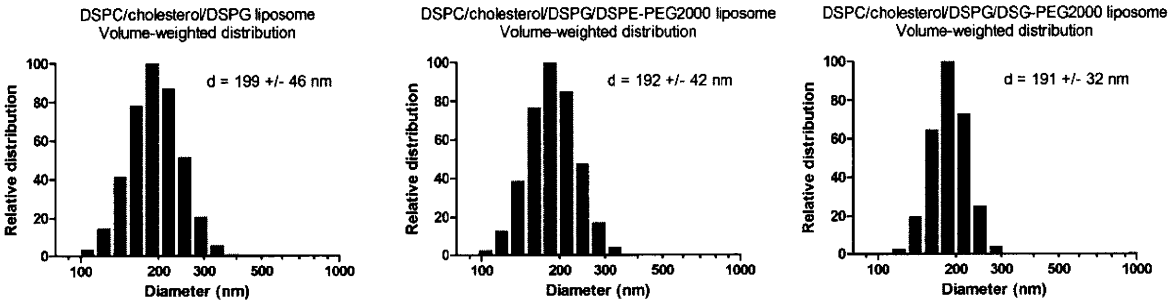

図 3　動的光散乱法による PEG 修飾リポソームの粒子径分布

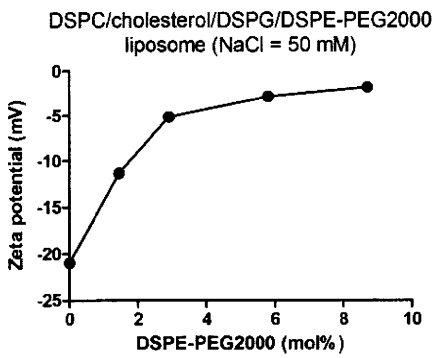

図 4　PEG 脂質濃度に依存した PEG 修飾リポソームのゼータ電位の変化（NaCl 濃度＝50mM）

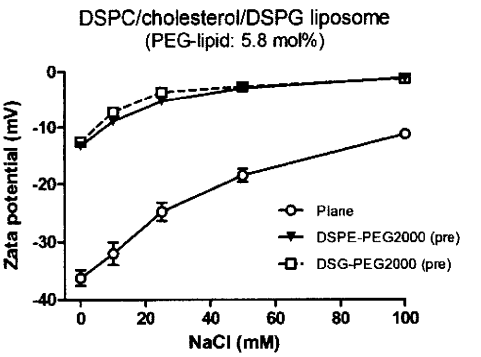

図 5　ゼータ電位の塩濃度依存性

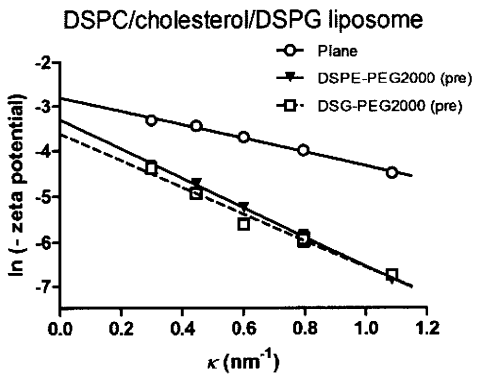

図 6　ゼータ電位とデバイパラメータとの関係

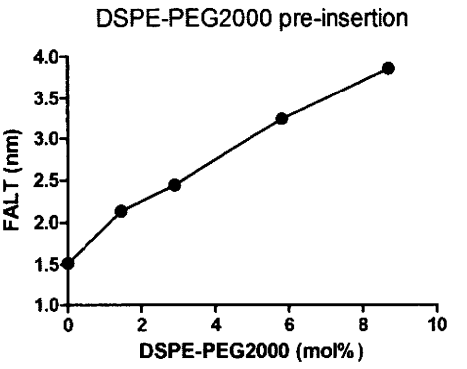

図 7　PEG 脂質濃度と固定水和層の厚さの関係

厚生労働科学研究費補助金(医薬品医療機器等レギュラトリーサイエンス総合研究事業)
後発医薬品の同等性ガイドラインにおける試験条件の最適化に関する研究
平成22年度 研究分担報告書

脂質分散系製剤の製剤評価法に関する研究(2)
—マイクロエマルジョン経口製剤に関する検討—

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研究要旨 脂質分散系製剤のうちシクロスポリンのマイクロエマルジョン経口製剤について,先発品と後発品とで各種溶液中(溶出試験溶液や人工腸液)における粒子径などの物理化学的性質を評価し,ラット経口投与後の体内動態との関連性を検討した.後発品は先発品よりも各種溶液に懸濁した際の濁度が顕著に高く,また明らかに粒子径が大きくなる傾向が認められ,先発品は約 40 nm だが後発品の中には100 nm前後になるものもあった.しかしながら,AUC や Cmax などに有意な差は認められなかった.従ってこの製剤に関しては,先発品と全く同じ粒子径でなくても 100nm 程度で均一に分散されていれば,体内動態が大きく異なる可能性は低いことが示唆された.ただし,Tmax は先発品よりも有意に遅くなることが認められ,詳細は不明であるが,物理化学的性質の差が影響しているものと推察された.

A. 研究目的

リポソームやリピッドマイクロスフェア,マイクロエマルジョンなど脂質分散系製剤の開発が活発に進められている.特に,シクロスポリンカプセルでは,従来の油性製剤を改良し,胆汁分泌量や食事の影響を少なくしたマイクロエマルジョン製剤が開発され,日本でも既に後発品4製剤が承認されている.しかし,生物学的同等性試験によって先発品との同等性が保証されているものの,先発品がマイクロエマルジョンという特殊な製剤であること,後発品に使用されている添加物は先発品と異なることなどから,その品質や有効性に不安が持たれているのが現状である.In vitro で評価できるマイクロエマルジョン製剤の重要な製剤特性としては,溶液に分散した際の粒子径や濁度が挙げられる.そこで,先発品と後発品4製剤を対象とし,カプセル内容物の懸濁液について粒子径や見た目の濁度を評価すると共に,この物理化学的性質の差が体内動態に与える影響を評価するため,ラットにおける体内動態を比較した.

B. 研究方法

(1)実験材料

シクロスポリンカプセルの試験製剤には以下のものを用いた;サンディミュンカプセル 50 mg (Product A, Lot S0016, ノバルティスファーマ), ネオーラルカプセル 50 mg (Product B, Lot S1046, ノバルティスファーマ), アマドラカプセル 50 mg (Product C, Lot 34006, 東洋カプセル), シクポラールカプセル 50 mg (Product D, Lot EC2501, 日医工), シクロスポリンカプセル 50 mg「マイラン」(Product E, Lot 0450RH, マイラン製薬), シクロスポリンカプセル 50 mg「FC」(Product F, Lot 9C1, 富士カプセル).なお,マイクロエマルジョン製剤の先発品は Product B, 後発品は Product C～F である.その他、油性製剤として Product A も評価対象とした.シクロスポリン A (CsA)の標準品は公定書協会より購入した.シクロスポリン D(CsD)は Enzo Life Sciences より購入した.

(2)試験溶液の調製

溶出試験第1液及び第2液は JP15 に準じて調製した.人工腸液の調製は Dressman らの報告[1]に従って調製した.以下に各人工腸液の組成を示す.FaSSIF(Fasted State Simulated Intestinal Fluid) : タウロコール酸 3 mM, レシチン 0.2 mM, マレイン酸 19.1 mM, 水酸化ナトリウム 34.8 mM, 塩化ナトリウム 68.6 mM (pH 6.5). FeSSIF (Fed State Simulated Intestinal Fluid) : タウロコール酸 15 mM, レシチン

3.75 mM, 塩化カリウム 203.8 mM, 酢酸 144 mM (pH 5.0).

(3)物理化学的性質の評価

カプセルに注射針で穴を開け, 内容物(液体)を取りだし, 各製剤にカプセル全体と空カプセルの重さを量り, 内容物の重量を算出した. 試験管に 80%相当量(重量)を量り取り, 各試験溶液 10 mL を添加し, 均一になるまで穏やかに転倒混和した. この溶液を原液とし, 別の試験管に5倍希釈, 25倍希釈の溶液を調製した.

粒子径の測定には動的光散乱法(DLS)を採用したDLS7000(大塚電子)を使い, レーザーは He-Ne, 散乱角度は 90℃で測定した. 100 回積算した値からヒストグラム及びキュムラント解析を行った. さらに, 蒸留水 10 mL で懸濁したカプセル内容物について, Accusizer (単一粒子光学検知法【SPOS】を採用) (Particle Sizing Systems)で粗大粒子数を測定した. 全て自動希釈率は 15 倍に設定し, 1 分間計数した. 棒グラフは 1 μm 以上の粒子数を示しており, 折れ線グラフは実測値を示している.

(4)ラット経口投与後の体内動態解析

一晩絶食させた SD ラット(♂ 300g, n = 5)に, カプセル内容物を CsA 1.0 mg/mL(3.5 mg/kg)となるように水 50mL に懸濁した溶液を胃ゾンデで経口投与した. EDTA を入れたシリンジで各時間に頸静脈より 300 uL 採血し, 分析に用いるまで-80℃にて保存した. 採取した全血 100 uL に内部標準溶液(CsD/メタノール)200 uL を添加し, 混和・遠心分離した後の上清をフィルターに通し, LC-MS の分析試料とした. 分析条件は Koseki らの報告[2]を参考に構築した. 体内動態パラメーター, AUC, Cmax 及び Tmax は WinNonlin(version 5.2, Pharsight Corporation, USA)を用いて計算した.

C. 研究結果

見た目の濁度と粒子径の比較

各製剤からカプセル内容物を取りだし, 試験液に懸濁する前の状態を比較したところ, 先発品Bはわずかに黄色いのに対し, 後発品 C・D は無色に近く, 後発品 F は先発 B よりも黄色く, 既に色が異なることが観察された(Fig. 1a). 各製剤のカプセル内容物1カプセル分を 10mL の蒸留水, 溶出試験第1液, 2液, 人工腸液 FaSSIF で懸濁したところ, 先発品 B と後発品 E は透明に近い水溶液となった. 一方, 後発品C・D・Fは濁りが認められ, 油性製剤である先発品 A とは状態が異なるものの, 後発品Fは白く, 後発品 C と D は青白く濁った. 人工腸液 FeSSIF で懸濁したところ, 後発品全て白濁したのに対し, 先発品 B はほぼ透明であった. (Fig. 1b)

カプセル内容物1カプセル分を 10mL の蒸留水に懸濁し, 0.5 μm 以上の粒子径分布と粗大粒子数を SPOS により測定したところ, 先発品 B と後発品 E がほぼ同程度の値であったのに対し, 後発品 C・Dには先発品 B の約 5 倍, 後発品Fには先発品 B の約25倍の粗大粒子数が含まれ, 見た目(Fig. 1)とほぼ相関した結果が得られた. (Fig. 2)

カプセル内容物を各試験溶液で希釈した際の平均粒子径と粒子径分布を DLS で測定した(Fig. 3, Table 1). 蒸留水中の平均粒子径は, 先発品 B 26.4 nm に対し, 後発品 4 製剤は C 74.8, D 64.5, E 39.7, F 79.2 nm と大きい傾向が見られた. 溶出試験第1液, 2液でもほぼ同様の傾向がみとめられたが, 後発品 F は溶出試験第2液や人工腸液 FaSSIF に懸濁すると粒子径が大きくなることが分かった. また, 人工腸液 FeSSIF に懸濁した後発品4製剤の平均粒子径(5倍・25倍希釈)は 100～200 nm と顕著に大きくなり, Fig. 1 の結果とおよそ相関していた.

体内動態の比較

CsA と代謝物(AM1, AM9, AM1c)の体内動態パラメーターを比較した(Fig. 4, Table 2). 先発品 B は油性製剤である先発品 A の吸収速度と吸収率を改良した製剤である. 先発品 B は先発品 A と比較して, CsA と代謝物の Tmax は顕著に早くなり, Cmax 及び AUC が有意に高くなることが確認できた. 後発品も有意な差はないものの, 先発品 A よりも Cmax や AUC が高くなる傾向が認められた. 後発品の CsA の Tmax は先発品 B よりも明らかに遅くなっていたが, Cmax と AUC に関しては先発品 B と後発品4製剤との間で有意な差は認められなかった. また, 代謝物の Cmax や AUC に関しても先発品 B と後発品4製剤との間で明

確な差は認められなかった.

以上の結果から、先発品Bと後発品4製剤で試験液に懸濁した際の粒子径や濁度に明らかな差は認められるものの、CsA やその代謝物の体内動態が大きく異なる可能性は低いことが示された。

D. 考察

水に分散させると先発品 B は透明な溶液になるのに対し，後発品では少し白濁する，全体的に青白く濁るなど，見た目の状態や粒子径が異なることが確認された．これは，後発品に使われている油や cosurfactant，cosolvent が先発品と異なるため，先発品 B と完全にマイクロエマルジョン状態にはならず，エマルジョンに近い状態，もしくはマイクロエマルジョンとの混合状態にあるためではないかと推察される．特に後発品Fは先発品Bと比較して粒子径が大きいだけでなく，粒子の分布が広く，粗大粒子数も多いため，エマルジョンが混在している可能性が高いと思われた．

このように，先発品と後発品とで物理化学的性質に明確な差が認められたにもかかわらず，ラット経口投与後の AUC や Cmax には大きな差は認められなかった．マイクロエマルジョンによる難溶性薬物の吸収改善メカニズムとしては，消化管内での薬物の溶解性を向上させることの他に，粒子径の小さい油滴を形成することで腸管細胞と相互作用する表面積を大きくできること，局所的に細胞膜へダメージを与え，膜透過性を向上させることなどがあげられる．このような作用は粒子径だけではなく粒子表面に存在する界面活性剤や cosurfactant などの性質にも寄与すると思われる．先発品 B と後発品では油や cosurfactant などが異なるものの，主要な界面活性剤は同じ種類のものが使われている．従って，先発品Bと後発品の AUC や Cmax に差が認められなかった理由として，先発品と後発品とで同じ種類の界面活性剤が使われていることと，ある程度小さい粒子径の均一な分散溶液となることから，AUC や Cmax に影響するほど薬物吸収部位での挙動に違いが無かったものと考えられる．

一方で，先発品 B と後発品全てにおいて Tmax に有意差が認められた．ラットは空腹時・食後関係なく常に胆汁酸が分泌されていると言われており，仮にラット腸内で FeSSIF に懸濁した溶液に近い状態であったとするならば，先発品 B だけが極めて小さい粒子径を保っていることから，Tmax の差は粒子径の差が反映した結果という可能性も考えられる．しかし，実際にラットの消化管内において各製剤がどのような状態であったか分からないため詳細は不明である．ただし，もしヒトにおいて今回のような Tmax の差があったとしても（ヒト BE 試験において Tmax に差は認められていない），先発品 B と後発品の近似 T1/2 の最大変化率は1割にも満たないので，投与設計に与える影響は少ないものと考えられる．

E. 結論

以上，先発品 B と後発品では見た目や粒子径等の物理化学的性質に明らかな違いがあるものの，少なくとも今回のラットの体内動態試験の結果は後発品の非同等性を示すものではなかった．粒子径が1～10μmの油性製剤である先発品 A よりも，粒子径が 100 nm 程度にコントロールされている先発品 B や後発品4製剤の AUC や Cmax は明らかに大きかったことから，シクロスポリンのマイクロエマルジョン製剤において粒子径は重要な要因の一つではある．しかし，先発品 B と後発品において，粒子径が小さいほど AUC が大きくなるというような相関性は認められなかった．従って，今回評価したシクロスポリンのマイクロエマルジョン製剤に関しては，生物学的同等性試験における粒子径評価の利用には少し制限があると考えられる．今後の製剤開発研究として，界面活性剤，補助界面活性剤や油がどのくらい CsA の体内動態に影響するのか詳細な検討が望まれる．

F. 参考文献

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