参考（ガイドライン英訳）

Guideline for the Development of Liposome Drug Products

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1. Introduction

A liposome is a microvesicle composed of a bilayer of lipid amphipathic molecules, and usually encloses an aqueous compartment. Liposome drug products are formed when a liposome is used to encapsulate an active substance within the lipid bilayer or in the interior space of the liposome. Many liposome drug products are designed to improve the stability of encapsulated active substances *in vivo*, the pharmacokinetics (including tissue distribution profile) of the active substances, and intracellular behavior of the active substances. Therefore, to establish a safe and effective dose and dosage regimen of a new liposome drug product, the characterization of its pharmacokinetics, including its tissue distribution profile, is essential.

An active substance in a liposome drug product exhibits different tissue and/or intracellular distribution profiles compared to those of the same active substance when administered by itself, due to the enhanced permeability and retention (EPR) effect or active targeting by the liposome modified with a ligand (targeting moiety) or antibody. Accordingly, the concentrations of active substances in the target tissue, cells, or intracellular organelle may differ between the dosage forms, even if the blood concentrations of active substances are similar. The pharmacokinetic data should, therefore, be carefully interpreted. In addition, as the target tissue and intracellular distributions are related to the quality attributes of the liposome, the physical, chemical, and biological properties of the liposome drug product (such as size and surface charge) also have to be evaluated. Furthermore, because liposomes are particles with diameter that can be readily recognized as foreign matter *in vivo* in many cases, it is critical to ensure appropriate *in vivo* stability in the design of the liposome.

The objective of this document is to facilitate the rational development of liposome drug products and their efficient regulatory review by identifying the points to be considered in the development of liposome drug products, and by presenting some examples required for the registration application.

2. Scope

This document applies to liposome drug products designed and manufactured to influence the stability of encapsulated active substances *in vivo*, and the pharmacokinetics (including tissue distribution profile) of the active substances, and intracellular distribution of the active substances. Although lipid-active substance mixtures and complexes in non-bilayer lipid form that are used to enhance encapsulation/solubilization/transportation of the active substance fall outside the scope of this document, the concepts described here would be helpful. This document mainly provides information regarding the pharmaceutical development and nonclinical and early clinical studies of liposome drug products. In addition, the principles outlined in this document are helpful to discuss post-marketing issues. Liposome drug products

described in this document are also subject to other relevant notifications and guidelines.

The active substances mentioned here include a low-molecular-weight chemical entity, a nucleic acid or a biological or biotechnological entity, including, for example, peptides and proteins.

3. Chemistry, manufacturing, and controls

Recommendations on the chemistry, manufacturing, and controls (CMC) described in this document focus on the specific aspects of liposome drug products. For general recommendations related to the quality of each active substance and excipient, refer to the relevant notifications and guidelines. Because the quality of liposome components such as lipids can affect the quality of whole liposome drug products, the quality of liposome components should be appropriately controlled, as described in Section 3.4.

3.1 Description and composition

Liposomes mainly consist of the active substance and lipid, but may contain functional lipids modified with, for example, polyethylene glycol (PEG) and/or ligand (targeting moiety). In addition, liposomal formulations contain excipients such as pH adjusters and stabilizers such as those found in general injections.

The following quality attributes are particularly important to define the properties of liposome drug products:

- Components of the liposome
- Quantities of the active substance and each lipid
- Molar ratio or percentage by weight of the lipid (including functional lipid) to the active substance

The formulation, including lipid composition, can largely affect the quality, pharmacokinetic and pharmacodynamic properties, and safety profile of liposome drug products. With this in mind, the formulation development of the liposome drug product should be explained and justified (see Section 3.2).

3.2 Formulation development and characterization

3.2.1 Pharmaceutical development

The quality and nonclinical and clinical profiles required to achieve the purpose of use of liposomal formulation and to meet the intended use of the liposome drug product should be clarified. The development study should be conducted to establish that the drug product design (including the dosage form and formulation), quality attributes, manufacturing, container closure system, and usage instructions is appropriate for the purpose specified in the

registration application, and information on the development study should be described as a pharmaceutical development.

Liposome drug products may contain a liposome without the active substance encapsulated or, for liposomes modified with certain molecules (e.g., PEG, a ligand [targeting moiety], or antibody) on the lipid bilayer, liposomes may lack those molecules or have degenerated molecules in certain concentrations. Therefore, liposome drug products should not be recognized as an assembly of a single liposome. Consequently, formulation development and evaluation of quality attributes of the liposome drug product should be performed as a whole drug product, and the appropriate ranges should be established to ensure the desired quality of the product, taking the nonclinical and clinical data of the relevant lots into consideration. In addition, liposome drug products are pharmaceutically complex, and the end product testing alone is sometimes insufficient to define quality. Therefore, it is highly recommended that appropriate pharmaceutical development is undertaken based on the Quality by Design (QbD) concept as outlined in Q8(R2) and Q11 guidelines in the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). To ensure consistent drug product quality, a control strategy should be established based on the critical quality attributes and relevant parameters, and then the analytical procedures and specifications should be defined. The choice of the liposome components and the composition and function of each component should be described in relation to the quality target product profile (QTPP) and the performance of the drug product (e.g., active substance release, targeting delivery). In addition, investigations implemented on the development study should be clearly explained in terms of how variations in the formulation development, quality attributes (physical, chemical, and biological properties), and manufacturing process would affect the performance of the drug product. Where necessary, the impact on the pharmacokinetics, efficacy and safety profile of the liposome drug products should be investigated and evaluated as well.

3.2.2 Characterization of drug products

To ensure the safety and efficacy of the liposome drug products, it is important to identify the critical quality attributes that would affect its *in vivo* pharmacokinetic and pharmacodynamic properties. As it is important to appropriately set the parameters of physical, chemical, and biological properties for these critical quality attributes in order to ensure the quality of the liposome drug product, multiple lots should be included in the detailed characterization and evaluated. For liposome drug products supplied as a lyophilized product for injection or an injectable powder, the reconstituted drug solution should be evaluated as

well.

Detailed physical, chemical, and biological characterizations will be useful in evaluating the impact of any changes in the manufacturing (see Section 3.7). For drug products containing molecules such as a protein and aptamer, whose conformation plays an important role in the function of the molecule as an active substance or molecules for liposome modification, the quality characterization, such as biological activities and immunological properties, should be identified and evaluated as the components and/or whole liposome drug product corresponding to the evaluation of biotechnological or biological products.

The following quality attributes are typical examples to be considered particularly for liposome drug products:

- Particle size distribution: The distribution should be expressed not only as the mean or median, but also in figures and as values of quantitative indicators, such as a polydispersity index. Determination of particle size is mainly performed by dynamic light scattering measurement, but for drug products with a large particle size, laser diffraction measurement is also used. If a dynamic light scattering measurement is used, it is important to select the appropriate distribution mode (e.g., count-base and/or volume-base distribution) and to clarify the selected mode.
- Morphology and/or structure of the liposome: The aggregation status and lamellar structure of the liposome should be examined. Image analysis techniques such as transmission electron microscopy, cryoelectron microscopy, atomic force microscopy, and small-angle X-ray scattering measurement can be used.
- Surface charge (zeta potential): Surface charge is usually a critical quality attribute because it has an impact on the *in vivo* clearance, tissue distribution, and intracellular uptake of the liposome. In general, the surface charge is evaluated as zeta potential, because it cannot be measured directly due to an electric double layer on the liposome surface formed by counter ions in the solution. Determination of surface charge is mainly performed by the electrophoretic light scattering (laser Doppler electrophoresis) method. As the zeta potential may vary depending on the compositions, pH, and electrical conductivity of the solvent used in the measurement, the testing conditions should be specified.
- Thermodynamic properties of the liposome membrane: The thermodynamic properties are evaluated by techniques such as differential scanning calorimetry and the temperature dependence of a fluorescence spectrum measured using a fluorescent probe for lipid membrane insertion. Thermodynamic properties such as exothermic and endothermic profiles are useful indicators of the fluidity and homogeneity of the lipid

bilayer membrane, even for liposomes whose definite phase transition temperature cannot be measured due to the presence of cholesterol or a highly lipophilic active substance.

- *In vitro* release characteristics of the active substance from liposome drug products: See Section 3.2.3.
- Osmolality: To prevent rupture or contraction of the liposome structure, the reconstituted drug solution should preferably be isotonic (approximately 280 mOsm/kg).
- pH: The pH of the dispersion fluid (external liquid phase) should be specified. If the properties and/or function of the liposome are expected to vary depending on a pH variation, an appropriate quality attribute should be identified for evaluation of the pH impact.
- Aggregation: As the aggregation and precipitation of liposomes may increase the incidence of the infusion reaction,[1] the aggregation status should be evaluated based on the appropriate test (e.g., turbidity measurement).
- Loading efficiency of the active substances: The active substance encapsulated in the liposome and the unencapsulated active substance are separated by solid-phase extraction, size exclusion chromatography, ultracentrifugation, gel filtration, or dialysis, and the amount of the active substance in each fraction is quantitatively measured by high performance liquid chromatography or a spectrophotometer.
- Impurities: Characterization of impurities should be made in accordance with the guidelines dealing with impurities in active substances and drug products (ICH Q3A, Q3B, Q3C, Q3D, and M7 guidelines). For liposome drug products in particular, material-related impurities, process-related impurities, product-related impurities (such as liposome aggregates and variants), and time-related degradation products should be carefully investigated, and the profiles of these impurities should be identified. The structure or forms of important impurities should be identified. When identification of an impurity is not feasible, a summary of the laboratory studies demonstrating the unsuccessful efforts to identify it should be included in the registration application.

The following additional characterization should also be considered depending on the characteristics of the liposome drug product:

- Physical state of the encapsulated active substance: The physical state of the encapsulated active substance should be characterized by electron microscopy or small-angle X-ray scattering measurement, especially in cases in which the physical state is critical in retaining the active substance (leakage of the active substance); for

[1] Szebeni J, et al. Adv Drug Deliv Rev. 2011;63:1020–1030.

instance, when the encapsulated active substance is present in the form of a gel by using the ammonium sulfate gradient method for encapsulation of the active substance.

- For drug products in which the liposome surface is modified with a targeting molecule such as a ligand, the (conformational) structure, modification efficiency, and binding capability of the modified liposomes to the target cells should be investigated, because the modification may affect the affinity of the liposome to the target cells. If these characterizations are difficult, they may be substituted with a biological assay.

3.2.3 *In vitro* release test

To ensure that the liposome drug product has a consistent *in vivo* stability and active substance release profile, an *in vitro* release test should be established using a test solution that appropriately reflects physiological conditions. Multiple release test conditions should be set depending on the characteristics of the liposome, drug product design, or intended use. The release of active substances from liposomes should be monitored using a physiologically and/or clinically relevant medium (e.g., buffer solution or human plasma), with suitable agitation where necessary. Even if the *in vitro* release profile of the active substance does not completely reflect the *in vivo* profile, a discriminatory *in vitro* release test should be developed and justified, taking the following aspects into consideration, where necessary:

- Release profile of the active substance from the liposome in blood and target tissue
- For liposomes that are designed to release the active substance in response to an environmental change (e.g., a pH change) in the target tissue or endosome, release profiles of the active substance from the liposome that reflects the physiological environment.
- For liposomes that are designed to release the active substance in response to a temperature change or external stimulation, release profiles of the active substances from the liposomes resulting from the response to the expected temperature change or external stimulation.

3.3 Manufacturing process and process controls of liposome drug products

To ensure the quality of liposome drug products, it is important not only to implement intermediate and process controls in the manufacturing process and to perform quality tests on the final product, but also to develop an appropriate control strategy based on the understanding of the manufacturing process. As previously described in Section 3.2.1, liposome drug products are sensitive to variations in manufacturing parameters. Therefore, the variation factors should be sufficiently understood by accumulating knowledge about the manufacturing process (e.g. process parameters and variability of raw materials) through the

development process.

Although the manufacturing of liposome drug products differs depending on the active substance to be encapsulated, the type and properties of lipid components, and the function and properties of the liposome drug product, the following are typical examples of manufacturing processes. The process conditions, product properties, and process control test should be set and controlled, where necessary, based on the control strategy.

3.3.1 Process of formation of liposomes

The process should be designed so that homogeneous liposome can be robustly manufactured in each manufacturing lot. The lipid composition in the liposome must be controlled to ensure consistency among the lots. In addition, process parameters that have an impact on the structure of the liposome should be identified, and appropriate control ranges should be set.

During the process wherein liposomes are manufactured using lipid thin membranes or lipid suspensions as manufacturing intermediates, it is important to ensure the homogeneity and consistency of these intermediates. The hydration time, mixing speed, and temperature of the hydration process can be critical process parameters.

For liposomes encapsulating macromolecules such as proteins, the formation of liposomes and encapsulation of the active substance take place at the same time. Therefore, it is important to ensure that the loading efficiency of the active substance is within a certain range during the process of this liposome formation. In the mixing process, the type of the solvent, ionic strength, mixing speed, and mixing temperature can be critical process parameters.

3.3.2 Encapsulation process of the active substance in liposomes

The process should be designed and controlled to ensure that the loading efficiency of the active substance is consistent between lots. If the active substance is encapsulated based on a pH gradient or a difference in the solubility, critical process parameters that affect the loading efficiency include, for example, pH and composition of the aqueous phases inside and outside the liposome and operating conditions (e.g., temperature, time).

If a removal process of active substances that were not encapsulated in the liposomes is included, the process capability should be evaluated, and the amounts of unencapsulated active substances should be controlled.

3.3.3 Sizing process

The pharmacokinetic behavior of liposome drug products is largely affected by the particle size. If the particle size distribution of the manufactured liposomes is large, particle sizing is

performed.

If size exclusion chromatography is used for purification, the type of the chromatography resin and column scale, liposome load, chromatography conditions, and fractionation method should be optimized, and the results of the optimization study should be provided from the viewpoint of, for example, separation capacity.

In addition, if particle sizing is performed by extrusion through membrane filters, parameters such as the lipid concentration, temperature, pressure, and filter pore size would be important. If the extrusion is repeated or carried out in a stepwise manner with multiple filters, conditions such as the number of extrusion operations, combination of the filters, and the order should be studied, and the results of the study should be provided.

3.3.4 Process for surface modification such as PEGylation

In some liposome drug products, the surface is modified with PEG to maintain its *in vivo* stability, or modified with a ligand (targeting moiety) or antibody to improve targeting delivery. This process should be designed and controlled to ensure that the modification status of the liposome is consistent between lots, considering the purpose of the surface modification and the modification efficiency required to exhibit the function. Considering the limitations of the final product testing, it is particularly important to adequately control the quality of the modified lipids and the manufacturing process, as described in Section 3.4.

3.3.5 Sterilization process

As liposomes are unstable under operating conditions used in dry-heat sterilization and autoclaving, sterilizing filtration is widely used for sterilization of liposome drug products. The process capability of sterilizing filtration should be assessed by a bacterial challenge testing to justify the filter selection.

3.4 Control of liposome components

In liposome drug products, lipid components forming the lipid bilayer and molecules for liposome modification such as PEG, ligands (targeting moiety), or antibodies, contribute to an improvement in the *in vivo* stability, pharmacokinetics, and intracellular behavior of the active substance. Therefore, liposome components, especially ligands (targeting moiety) and antibodies that have a significant impact on the function of the drug product, should be evaluated and controlled to a greater extent than general excipients to ensure their intended properties.

3.4.1 Quality attributes

If the lipid is synthetic and expressed in a single structural formula, its structure should be generally elucidated by standard spectroscopic techniques. If the lipid is a natural mixture (e.g., soy lecithin and egg lecithin) or semisynthetic (e.g., hydrogenated soybean phosphatidylcholine [HSPC]), its lipid composition (i.e., percentage of each lipid) and the composition of fatty acid of lipid (i.e., percentage of each fatty acid) should be clarified, because the properties of the liposome drug product may change due to variations in lipid composition.

Lipids modified with polymers such as PEG or molecules for targeting delivery (e.g., ligands [targeting moieties]) should be structurally characterized, including the linker. The molecular weight distribution of PEG is particularly important, because it may have an impact on not only the *in vivo* stability of the liposome, but also on its particle size and release of the active substance.

3.4.2 Manufacturing process and process control

For synthetic and semisynthetic lipids, the starting material and manufacturing should be specified and appropriately controlled. Regarding the biological source used for the synthesis of semisynthetic lipids, the biological source (e.g., eggs) and supplier should be provided. If a synthetic and semisynthetic lipid is manufactured by the applicant or has been contracted, the specifications of the starting material, as well as the controls for the critical process and intermediates, should be defined, and the process parameters that have an impact on the quality of the lipid should be provided.

If a biological entity or biotechnology-derived recombinant protein is used as the starting material or raw material, or is directly used in the liposome drug product, it should be controlled and investigated in accordance with the requirements of the other notifications and guidelines relevant to such biotechnological/biological products.

3.4.3 Specifications

The impact of components of the lipid bilayer such as lipids and ligands (targeting moiety) on drug product quality should be investigated. Based on the study results, the tests should be adequately set, and the analytical procedures and specifications should be provided in detail. As the excipients should have properties that suit the intended use of the liposome drug product, even for those listed in the compendia, the analytical procedures and acceptance criteria may have to be additionally set and controlled in the following cases: where the properties relevant to drug product quality are not specified in the compendia, or where the intended drug product quality cannot be ensured by the control standard defined in the compendia. Validated analytical procedures should be used, and the specifications should

include at least the content (or strength) of the component, its identification, purity test, and an assay. To establish the reference standard or reference material, the preparation method, specifications, and analytical procedures, as well as the storage conditions and shelf life, should be described.

If a biological or biotechnological entity such as a protein is used, the specifications should be appropriately defined in accordance with the ICH Q6B guideline.

3.4.4 Stability

Lipids, especially those modified with a ligand (targeting moiety), should be sufficiently stable, as they are required to exhibit special functions compared with the general excipients in the drug product. The stability of such lipids should be appropriately evaluated in accordance with the concepts in the ICH Q1A(R2) guideline and/or ICH Q5C guideline to establish the retest date or shelf life.

3.5 Control of liposome drug products

3.5.1 Specifications

The analytical procedures and acceptance criteria should be set based on the Japanese Pharmacopeia and ICH Q6A or Q6B guidelines. In addition, tests specific to liposome drug products (e.g., loading efficiency of the active substance, release rate of the active substance, assay of lipid components, and degradation products) should be appropriately established where necessary. For the quality attributes that can change over time, acceptance criteria should be established, taking into consideration the effects on the pharmacokinetics, efficacy, and safety of the liposome drug product, and then the established acceptance criteria should be justified. Validated analytical procedures applicable to the liposome drug product should be established. These tests and the analytical procedures should be based on the properties selected to characterize the drug product, including those listed in Section 3.2.2, as appropriate. In particular, the following points should be considered in establishing the specifications for a liposome drug product.

3.5.2 Identification

The analytical procedures and acceptance criteria should be established, so that they can comprehensively ensure, in combination with the other specification tests, that liposome components such as lipids and molecules for liposome modification constitute the liposome appropriate for the purpose specified in the registration application.

3.5.3 Endotoxin test

Some liposome drug products are not compatible with the endotoxin test, failing to provide accurate values due to a cross-reaction between the lipid and lysate reagent.[2] Therefore, validation of the endotoxin test should be appropriately implemented. If the endotoxin test is not appropriately performed, the endotoxin should be controlled by the pyrogen test.

3.5.4 Biological assay

The biological activities of the components and/or the whole drug product should be evaluated where necessary, if the liposome drug products contain molecules such as a protein and aptamer, whose conformation plays an important role in the function of the molecule, as an active substance or a molecule for liposome modification.

To perform a biological assay with a liposome drug product, the reference material for the liposome drug product may have to be established. To establish the reference material, the manufacturing as well as the analytical procedures and acceptance criteria should be provided. To evaluate the stability of the reference material, characterization tests should be appropriately selected, and consistent quality attributes of the reference material throughout the shelf life should be ensured.

3.6 Stability

The stability study of liposome drug products should be conducted in accordance with the ICH Q1A(R2) guideline. If a biotechnological/biological product (biological entity or biotechnology-derived recombinant protein) is used as the active substance or liposome component, the concepts in the ICH Q5C guideline are also applied. Because the current state of knowledge on the stability of liposome drug products is limited, establishing the shelf life beyond the stability period confirmed via long-term stability testing is basically not acceptable.

As liposome drug products are pharmaceutically complex, stability testing should include not only those test items in the specification, but also additional specific characterization tests, where necessary, to thoroughly understand the changes in quality attributes over time. The following properties may be evaluated as important tests specific to liposome drug products:

- Stability of each lipid component in the liposome drug product:

  Lipids with unsaturated acyl chains are subject to oxidative degradation, which then causes changes in phase transition temperature, consequently affecting the stability of the liposome. Both saturated and unsaturated lipids are subject to hydrolysis to form lysolipids and free fatty acids. Degradation of the lipids can deprive liposome drug products of their original function or lead to disintegration of the lipid bilayer structure. Therefore, it is important to clarify the extent of degradation of the lipids and the impact

[2]Dobrovolskaia MA, et al. Nanomedicine (London). 2010;5:555–562.

on the quality attributes of the liposome drug product.

- Modification efficiency with molecules and their structural stability:
  Depending on the binding modality of PEG and other molecules for modification of liposome, those molecules can be gradually disassociated, leading to a decrease in modification efficiency. In addition, conformational changes may occur in PEG or other molecules for liposome modification due to long-term storage, depending on the type of aqueous phase outside of the liposome or the storage condition. If a decrease in modification efficiency or conformational change is observed, the impact on the quality attributes of the liposome drug product should be clarified.
- Particle size distribution and aggregation:
  Liposomes are susceptible to fusion or aggregation during long-term storage. For instance, small unilamellar vesicles are susceptible to an increase in particle size as a consequence of fusion of the vesicles. Therefore, tests such as turbidity and particle size distribution should be set to evaluate these changes over time and the impact on the quality attributes of the liposome drug product.
- Loading efficiency:
  Leakage of the encapsulated active substance may be observed, irrespective of whether the lipid bilayer structure of the liposome is disintegrated or intact. Therefore, the changes of the loading efficiency over time and the impact on the quality attributes of the liposome drug product should be evaluated.

3.7 Changes in the manufacturing

Because liposome drug products are different from general low-molecular-weight chemical drug products, it is not possible to provide recommendations on the standard dataset to demonstrate that the change has not adversely affected the quality of the drug product.

In addition to the specifications for the drug product, tests relevant to the physical, chemical, and biological properties that may be affected by the change in manufacturing should be identified, taking into consideration the properties of the liposome drug product, the control strategy, and the type of change. Using those tests, the liposome drug products should be evaluated to confirm that the quality before and after the changes made in the manufacturing is comparable. The concepts outlined in the ICH Q5E guideline (Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process) should be considered for assessing the comparability of liposome drug products before and after changes are made in the manufacturing. If the extent of the change is significant and the data on quality attributes are unlikely to assist in determining whether pre- and post-change product are comparable, or if comparability of the drug product quality cannot be explained

based on the physical, chemical, and biological properties, the impact of the change on the pharmacokinetics, efficacy, and safety of the liposome drug product should be evaluated and investigated, taking the content in the Appendix into account.

The properties of liposome drug products are considered to be more sensitive to the scale-up than those of conventional low-molecular-weight drug products. Therefore, scale dependency should be carefully evaluated. For instance, a scale-up of the extrusion process for sizing of the liposome may involve changes in the pressurization method and filter type. A scale-up of the purification process using size exclusion chromatography will involve a change in the column scale, which in turn requires a change in the fractionation condition (e.g., flow rate and liposome load). The relationship of these process changes with the quality attributes of the liposome drug product should be thoroughly investigated to confirm that the quality of pre- and post-change liposome drug products is comparable.

Changes relevant to the manufacturing principle, the loading method of the active substance, the weight ratio of lipids to the active substance, composition of the lipid bilayer, and surface modification process of the liposome could affect the efficacy and safety of the liposome drug product, and such changes will require special consideration.

A change in the manufacturing after confirmatory trials requires a comparability assessment of the liposome drug products as comprehensively and thoroughly as those required for the post-approval change.

4. Nonclinical studies

4.1 Introduction

Significant changes in pharmacokinetic characteristics can occur when an active substance is administered as a liposome drug product from those of the active substance administered by itself (i.e., changes in distribution volume and clearance, extension of the half-life, or a change in *in vivo* distribution may occur). Consequently, significant differences not only in the pharmacokinetic characteristics but also in the efficacy and safety of the active substance can be observed when the active substance is administered as a liposome drug product.

After being delivered to the tissue, the liposome usually exhibits the pharmacodynamic response through the following processes: the liposome is incorporated into the cells and then releases the active substance, or the active substance is extracellularly released from the liposome and then incorporated into the cells.

In general, the pharmacokinetic characteristics of the liposome drug product could be dependent on:

- Clearance of the liposome encapsulating active substance
- Release rate of the encapsulated active substance from the liposome

- Distribution of the liposome (changes in organ and/or tissue distribution and the amount of distributed active substances)
- Interaction of the liposome or active substance with plasma or serum protein, blood cells, or vascular endothelium

The rate and location of *in vivo* active substance release is a crucial parameter, which often determines the pharmacological effect and safety. An attempt should be made to develop the necessary methodology to understand the active substance release profile.

Nonclinical studies should be conducted using a well-characterized liposome drug product equivalent to the drug product for clinical use, and the release rate of active substance and product stability should be known under the chosen test conditions.

4.2 Nonclinical pharmacokinetics

4.2.1 Analytical methods

Analytical techniques should be developed that are capable of measuring the concentrations of active substances (in total and unencapsulated forms and where necessary encapsulated form) in blood, plasma or serum, and the total concentration of active substance in organs and/or tissues. Depending on the properties of the liposome drug product (e.g., release properties of active substances *in vivo*), it may be important to measure the concentrations of active substances encapsulated in the liposome. If a liposome component is predicted to affect safety, analytical techniques that are capable of measuring the concentration of liposome components should be developed.

The concentrations of active substances that are not separated into the encapsulated and unencapsulated forms in the blood, plasma, or serum sample at each time point after administration should be measured as the "total concentration," along with the unencapsulated active substance concentration. Although the concentrations of the active substance in the unencapsulated forms as well as in total in the blood, plasma, or serum can be measured, measurement of the unencapsulated active substance concentration in organs and/or tissues may be difficult because the liposome can disintegrate during sample processing. Careful attention should be paid to appropriately control the sample processing procedures during the course of method development in order to verify the suitability and interpretability of the obtained bioanalytical results.

The analytical technique used to measure the active substance concentration (in total and unencapsulated forms and where necessary encapsulated form) in the blood, plasma, or serum, and concentration of the total active substance in organs and/or tissues and metabolites should be validated, and such validation practices should be described. The references for such validation may include the PFSB/ELD Notification No. 0711-1 "Guideline for validation of

bioanalytical methods in drug development" (dated July 11, 2013) and PFSB/ELD Notification No. 0401-1 "Guideline for validation of bioanalytical methods (ligand-binding method) in drug development" (dated April 1, 2014).

4.2.2 Pharmacokinetics

The pharmacokinetic behavior of a liposome drug product can be largely different from that of the active substance administered in a non-liposomal form, and this difference may have a remarkable impact on the efficacy and safety of the product. It is therefore important to compare the *in vivo* pharmacokinetics of the active substance administered by itself and the liposome drug product. When the *in vivo* pharmacokinetics and active substance release are investigated, the selection of animal species and animal model should be justified, with careful consideration of the following points: the expected clinical application of the liposome drug product, liposome composition, the properties of the active substance, and blood concentration and tissue distribution including the accumulation and retention in the target organ and/or tissue of both the active substance and liposome drug product. If ligands (targeting moiety) or antibodies are conjugated to the liposome surface to provide targeting delivery, the animal species and model should be selected considering the differences in the expression and distribution of the receptor or epitope between the selected animal species and humans.

As the quality attributes of liposomes such as the size, surface charge, morphology, and surface modification with a ligand (targeting moiety) or antibody may affect the *in vivo* distribution of a liposome drug product, the impact of variations in such properties on the *in vivo* distribution should be assessed. Investigating the relationship between the quality attributes and *in vivo* distribution will help justification of the product specifications in the future. In addition to the recommendations in the ICH S3 (S3A and S3B), S6(R1), and M3(R2) guidelines, the following factors are important in assessing the liposome drug product:

- It is useful to explain the purpose and significance of the liposome formulation by comparing the pharmacokinetics of the liposome drug product and the active substance administered by itself.
- The appropriate pharmacokinetic parameters such as the Cmax, area under the curve (AUC), and half-life of the total active substances and unencapsulated active substance in the blood, plasma, or serum should be analyzed, and changes in the pharmacokinetics of the active substance due to the liposome formulation should be discussed.
- The pharmacokinetic parameters should be measured at different dose levels and at appropriate time points.
- Distribution of the liposome drug products in organs and/or tissues relevant to proposed clinical use and route of administration should be evaluated. Specifically, total amounts

of active substance in organs and/or tissues are required. A distribution time profile should be obtained using adequate sampling time points and sampling duration so as to accurately quantify the time course of the active substances.

- Some factors should be considered for the sampling schedules, such as sampling time points and sampling duration (e.g., the liposome stability after administration, and the profile of localization to specific organs and/or tissues). In particular, samples taken in the initial distribution phase (e.g., <15 min) are considered informative for calculating the distribution volume to estimate the stability of liposome in blood circulation (i.e., stability related to the initial burst of the liposome).
- If data on the concentration of the unencapsulated active substance in the relevant organs and/or tissues with regard to the safety and efficacy of the liposome drug product are not available on account of difficulties in the analytical technique, attempts to measure the metabolites are useful.
- Study design details such as sampling method and sampling time points will affect precision of derived parameters. The appropriate dose levels, necessary sampling schedule, and the number of animals should be carefully determined.
- It is desirable to analyze the distribution of liposome drug product in organs and/or tissues associated with the safety and efficacy of the liposome drug product, as well as those involved in major metabolism and elimination of liposomes. Organs with safety concerns include the reticuloendothelial system, important organs related to clearance, and organs with accumulation potential (e.g., liver, spleen, kidneys, bone marrow, lungs, and heart), as well as organs protected by a blood-tissue barrier (e.g., the brain and testes).
- Measurement of active substance metabolites in blood, plasma, or serum (and also the organs and/or tissues, if possible) is especially important when the metabolite is acknowledged to be the primary active compound. If one or more metabolites have substantial clinical activity, it is recommended to compare their pharmacokinetics and, where necessary, toxicokinetics, to determine accumulation following multiple doses.
- It may also be important to consider the protein and cellular interactions of intravenously administered liposome because this factor is known to have the potential to influence the distribution, stability, and safety of liposome drug products.
- It is also useful to understand the pharmacokinetic behavior of the liposome drug product using an appropriate animal model and imaging technique.
- A ligand (targeting moiety) or antibody on the liposome surface can have a substantial impact on the tissue distribution and intracellular distribution of the liposome. It should be noted that these modifications can change the accumulation of liposome drug product,

not only in target organs and/or tissues, but also in the other organs and/or tissues.

- The metabolic and excretion pathways of the active substance of a liposome drug product should be evaluated, because such an evaluation is linked to the safety and efficacy evaluation of the drug product. If a liposome component is predicted to affect safety, the distribution, metabolic, and excretion pathways of the component should be evaluated, where necessary.

4.3 Nonclinical pharmacodynamics

The nonclinical pharmacodynamic studies should include demonstration of pharmacodynamic response in appropriately justified *in vitro* (where possible) and *in vivo* models. *In vivo* evaluation should involve an appropriate route of administration, justified dose levels, and a justified dosing regimen, depending on the proposed clinical application. Appropriateness of the pharmacological model should be discussed in respect of the pharmacokinetic behavior of the liposome drug product, as well as the pharmacokinetics and pharmacodynamics of the active substance when administered by itself.

The chemical composition and physicochemical properties (including size, surface charge, and the release rate of the active substance) of a liposome drug product affect pharmacodynamic properties. Some important factors to consider when designing studies to discuss the mechanisms of action include:

- The location and rate of *in vivo* active substance release.
- The binding of the liposomes to the target cells if a ligand (targeting moiety) or antibody is conjugated to the liposome surface.
- The intracellular fate of the liposomes (including lipids or other components) following cellular entry by endocytosis or other mechanism, if the intracellular release of the active substance plays an important role in exhibiting the pharmacodynamic effect.

The pharmacodynamic effect of the liposome drug products should be assessed using *in vitro* (where possible) and *in vivo* pharmacodynamic models. The development of *in vitro* tests capable of characterizing any interaction between liposomes and target cells is encouraged. If a ligand (targeting moiety) or antibody is conjugated to the liposome surface, the pharmacological action derived from the ligand (targeting moiety) or antibody should be determined in addition to the affinity to the target cells. Failure to use both *in vitro* and *in vivo* models to assess the pharmacodynamic effects of the liposomes should be extensively justified using the evaluation method and the result by the applicant.

4.4 Safety pharmacology

For liposome drug products (e.g., those that fall outside the scope of ICH S9 and require the

safety pharmacology evaluation), safety pharmacology studies should be conducted in accordance with ICH M3(R2), ICH S7A, and ICH S7B, and in consideration of Section 4.5.

4.5 Toxicology

In principle, the nonclinical evaluation of toxicities of liposome drug products should be equivalent to the evaluation for drug with new active ingredients. The toxicity studies of the liposome drug product should be conducted to assess the toxicological profile and exposure-response relations according to the ICH safety guidelines and M3(R2) guideline in consideration of the following points:

- If a toxicity evaluation of the active substance administered by itself has already been completed, the toxicity of the liposome drug product using the same clinical route of administration as the active substance administered by itself should be evaluated by means of a short-term repeated-dose toxicity study using the intended clinical route of administration in one animal species. The obtained toxicity profile and toxicokinetic data should be compared with those of the active substance administered by itself. Based on the results, studies necessary for toxicity evaluation of the liposome drug product should be conducted from the generally conducted toxicity studies for drugs with new active ingredients.
- When the active substance is novel and toxicity and toxicokinetic data are unavailable, toxicity and exposure evaluations should be performed for the liposome drug product based on the ICH nonclinical safety guidelines. When the active substance is likely to be present in blood circulation in the unencapsulated form, it may be necessary to perform repeated-dose toxicity studies of the active substance alone in appropriate animal species, using the intended clinical route of administration, and to compare the obtained toxicity and toxicokinetic data with those of the liposome drug product.
- Safety evaluation of the liposome components as excipients can be performed with the complete drug formulation (the whole liposome drug product) if the intention is to have the components approved exclusively for that drug product. However, a toxicity evaluation of the components alone may be required when a suitable toxicity evaluation derived from the liposome components cannot be performed by using only the whole liposome drug product (e.g., because of novel toxicity concerns derived from the lipid structure or the potential for accumulation of the liposome components).

4.5.1 Toxicokinetics

In addition to blood, plasma, or serum concentration, measurement of the active substance in the target organs and/or tissues and toxicologically relevant organs and/or tissues is useful for toxicity evaluation of liposome drug products.

4.5.2 Additional studies

Depending on the physicochemical and/or pharmacokinetic characteristics of the liposome drug product and/or the lipids used for its manufacture, histological and functional evaluation of target organs may be necessary.

Acute infusion reactions are relatively common with liposome drug products. The use of *in vitro* and *in vivo* studies such as complement activation assays (and/or macrophage/basophil activation assays) and studies in appropriate animal models should be considered in order to evaluate the potential adverse events.

Studies to investigate hematotoxicity, antigenicity, and/or immunotoxicity (ICH S8) should be considered depending on the characteristics of the liposome drug product, including the characteristics of the liposome or the pharmacological properties of the active substance.

5. Considerations for first-in-human studies

Liposome drug products are often designed to influence the stability of encapsulated active substances *in vivo*, the pharmacokinetics (including tissue distribution profile) of the active substances, and intracellular distribution of the active substance. Therefore, in addition to the information recommended in the ICH S3 (S3A and S3B), S6(R1), M3(R2), and the PFSB/ELD Notification No. 0402-1 "Guidance for establishing safety in first-in-human studies during drug development" (dated April 2, 2012), when considering the first-in-human studies, it will be essential to consider information specific to the liposome drug product (e.g., nonclinical pharmacokinetic data of the liposome drug product and the active substance, proposed clinical use, and route of administration).

In a nonclinical pharmacokinetic study, the time course of liposome drug products for the total active substance, unencapsulated active substance, and metabolites (and encapsulated active substance, depending on the properties of the liposome drug product) should be quantified before first-in-human studies conducted using pharmacokinetic parameters, sampling time points and durations that have been carefully selected, as follows:

- Pharmacokinetic parameters such as Cmax, AUC, and half-life, both for the total active substances, and for unencapsulated active substances in the blood, plasma, or serum.
- A sufficient number of samples should be collected to adequately describe the plasma concentration-time profile. Frequent sampling at early time points is considered useful for providing reliable information about the initial distribution process. In general, the sampling schedule should be designed to provide a reliable estimate of the total extent of exposure.
- Distribution of liposome drug products in target lesions and major organs. During evaluation, the total amount of the active substance in the target lesion and major organs

should be measured at the time points that enable the estimation of the plasma concentration time profile over an adequate period of time.

The starting dose for first-in-human studies should be chosen in compliance with ICH M3(R2) and "Guidance for establishing safety in first-in-human studies during drug development," and by considering all related nonclinical data, including critical product attributes, pharmacological dose-response, pharmacokinetics, and pharmacological/toxicological profile as discussed in Section 3 (Chemistry, manufacturing, and controls) and Section 4 (Nonclinical studies) above.

Dose-limiting toxicity in humans can be determined in a similar way to that of conventional drugs, except for hypersensitivity reactions, because these reactions are not always dose dependent.

Potential critical quality attributes for each liposome drug product should be identified and used to evaluate consistency as discussed in Section 3. Consistency of the quality attributes should be confirmed between the products used for the first-in-human studies and those for nonclinical studies, and test procedures should be established before the commencement of first-in-human studies. If the manufacturing process (including the scale-up) used to prepare a liposome drug product for nonclinical studies is changed before the first-in-human studies are conducted, comparability should be demonstrated.

The stability of the liposome drug product must be ensured throughout the first-in-human studies by using the stability test.

6. Glossary

The purpose of this glossary is to describe terms as they are used in this document.

1) EPR effect: When a nanoparticle or high molecular protein that would not usually leak through an intact blood vessel is steadily circulated in the blood without being trapped by the reticuloendothelial system, the molecule preferentially leaks into solid tumors and accumulates there due to its microvascular hyperpermeability and impaired lymphatic drainage. This phenomenon is called the EPR effect.

2) Liposome: A liposome is a microvesicle, composed of a bilayer of lipid amphipathic molecules enclosing an interior compartment. Liposome drug products are formed when a liposome is used to encapsulate an active substance within the lipid bilayer or in the interior space of the liposome.

3) Loading efficiency of the active substance: (Amount of the active substance encapsulated in the liposome/amount of the total active substance in the drug product) × 100 (%).

4) Unencapsulated active substance: An active substance present in the drug product that is not incorporated within the lipid bilayer or in the interior space of the liposome. In this guideline, the term "unencapsulated" does not suggest the disassociation of active substances from plasma or serum proteins. The concentration of active substances that are not separated into the encapsulated active substances within the lipid bilayer or in the interior space of the liposome and unencapsulated active substances is referred to as the "total concentration."

5) Initial burst of liposome: The release of active substances from liposome drug product immediately after intravenous administration of a liposome drug product before being delivered to the target organs and/or tissues.

Related Guidelines

- ICH Harmonised Tripartite Guideline Stability testing of new drug substances and products Q1A(R2) [June 3, 2003, PMSB/ELD Notification No.0603001]
- ICH Impurities in New drug Substances Q3A(R2) [December 16, 2002, PMSB/ELD Notification No.1216001]
- ICH Impurities in New drug Products Q3B(R2) [June24, 2003, PMSB/ELD Notification No.0624001]
- ICH Impurities: Guideline for Residual Solvents Q3C(R3) [March 30, 1998, PMSB/ELD Notification No.307]
- ICH Guideline for Elemental Impurities Q3D [September 30, 2015, PFSB/ELD Notification No. 0930-4]
- ICH Quality of Biotechnological Products: Stability Testing of Biotechnological/biological product Q5C [January 6, 1998, PMSB/ELD Notification No.6]
- ICH Note for Guidance on Comparability of Biotechnological/Biological Products Subject to Changes in their Manufacturing Process Q5E [April 26, 2005, PFSB/ELD Notification No.0426001]
- ICH Quality of biotechnological/biological products Q5A(R1)-Q5D [February 22, 2000, PMSB/ELD Notification No.329 (Q5A(R1)), January 6, 1998, PMSB/ELD Notification No.3 (Q5B), and July 14, 2000, PMSB/ELD Notification No.873 (Q5D)]
- ICH Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances Q6A [May 1, 2001, PMSB/ELD Notification No.568]
- ICH Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products Q6B [May 1, 2001, PMSB/ELD Notification No.571]
- ICH Pharmaceutical Development Q8(R2) [June 28, 2010, PFSB/ELD Notification No.0628-1]
- ICH Development and Manufacture of Drug Substances (Chemical Entities and Biotechnological/ Biological Entities) Q11 [July 10, 2014, PFSB/ELD Notification No.0710-9]
- ICH Note for Guidance on Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies S3A [July 2, 1996, PMSB/ELD Notification No.443]
- ICH Pharmacokinetics: Guidance for Repeated Dose Tissue Distribution Studies S3B [July 2, 1996, PMSB/ELD Notification No.442]

- ICH Duration of Chronic Toxicity Testing in Animals (Rodent and Non rodent Toxicity Testing) S4 [April 5, 1999, PMSB/ELD Notification No.655]
- ICH Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals S6(R1) [March 23, 2012, PFSB/ELD Notification No.0323-1]
- ICH Safety Pharmacology Studies for Human Pharmaceuticals S7A [June 21, 2001, PMSB/ELD Notification No.902]
- ICH The Non-clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals S7B [October 23, 2009, PFSB/ELD Notification No.1023-4]
- ICH Immunotoxicology Studies for Human Pharmaceuticals S8 [April 18, 2006, PFSB/ELD Notification No.0418001]
- ICH Nonclinical Evaluation for Anticancer Pharmaceuticals S9 [June 4, 2010, PFSB/ELD Notification No.0604-1]
- ICH Safety [April 14, 1997, PMSB/ELD Notification No.315 (S1A), July 9, 1998, PMSB/ELD Notification No. 548 (S1B), November 27, 2008 PFSB/ELD Notification No. 1127001 (S1C(R2)), September 20, 2012, PFSB/ELD Notification No.0920-2 (S2(R1)), August 10, 1993, PMSB/ELD Notification No.88 (S4), April 5, 1999 PMSB/ELD Notification No.655 (S4A), April 14, 1997, PMSB/ELD Notification No.316 (S5A, S5B), December 27 2000, PMSB/ELD Notification No.1834 (S5B(M)), May 21, 2014, PFSB/ELD Notification No.0521-1 (S10)]
- ICH Pharmacovigilance Planning E2E [September 16, 2005, PFSB/ELD Notification No.0916001 and PFSB/SD Notification No.0916001]
- ICH Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals M3(R2) [February 19, 2010, PFSB/ELD Notification No.0219-4]
- ICH Assessment and Control of DNAreactive (Mutagenic) impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk M7 [November 10, 2015, PSEHB/ELD Notification No.1110-3]
- Guidelines for Non-clinical Pharmacokinetic Studies [June 26, 1998, PMSB/ELD Notification No. 496]
- Clinical Pharmacokinetics Studies on Drugs [June 1, 2001, PMSB/ELD Notification No. 796]
- Guidance for Establishing Safety in First-in-Human Studies during Drug Development [April 2, 2012, PFSB/ELD Notification No. 0402-1]
- Guideline for Validation of Bioanalytical Methods in Drug Development [July 11, 2013, PFSB/ELD Notification No. 0711-1]

- Joint MHLW/EMA Reflection Paper on the Development of Block Copolymer Micelle Medicinal Products [January 10, 2014, PFSB/ELD Notification No.0110-1]
- Guideline for Validation of Bioanalytical Methods (Ligand-Binding Method) in Drug Development [April 1, 2014, PFSB/ELD Notification No. 0401-1]

Appendix
Comparability assessment of liposome drug products subject to changes in their manufacturing

The comparability of liposome drug products before and after the change in manufacturing can be determined based solely on quality considerations, if the applicant can ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product through analytical studies, as suggested in this document (see Section 3 [Chemistry, manufacturing, and controls]). Additional evidence from nonclinical or clinical studies is required when quality data are insufficient to establish comparability.

A1. Nonclinical studies

For details on nonclinical studies, see Section 4 (Nonclinical studies). The combination of nonclinical studies for comparability assessment will be determined on a case-by-case basis, taking various factors into consideration, including: the extent of the change in manufacturing, the development stage, the complexity of the liposome drug product, and pharmacological properties.

A1.1 Nonclinical pharmacokinetics

Some of the pharmacokinetic properties of the liposome drug product in humans may be predicted based on the data from animal models, as well as *in vitro* cell culture models. The choice of animal species and model should be justified considering the purpose, nature, and extent of the change in manufacturing, as well as the impact on the quality attributes and pharmacokinetic properties. In addition, study conditions such as the dose levels, dosing schedule, and measurement parameters (pharmacokinetics of the total active substance and unencapsulated active substances and where necessary encapsulated active substances) should be justified from the viewpoint of the nature of the changes in manufacturing as well as the impact on the quality attributes and pharmacokinetic properties.

For instance, changes in the manufacturing or manufacturing process such as lipid hydration, blending of lipids, and sizing of the liposome can have an impact on the size, size distribution, morphology (including aggregation), and *in vitro* release rate of the liposome drug product. For liposome drug products whose surface is modified with a ligand (targeting moiety) or antibody, such changes can have impact on the modification efficiency. A detailed comparison of the quality attributes between the liposome drug products before and after these changes shows some similarity, but fails to reach a conclusion in terms of the impact on drug safety and efficacy due to, for example, limitations of the analytical procedures used. In such situations,

nonclinical pharmacokinetics studies comparing organ and/or tissue distribution and elimination should be considered in addition to the comparison of the blood concentrations and pharmacokinetic parameters.

A1.2 Nonclinical pharmacodynamics

It is recommended that *in vitro* studies be developed to characterize interactions of the liposome with target cells as well as with other cells of toxicological concern, if possible. Because it is recognized that the current state of knowledge on *in vitro* studies is limited, similarities in pharmacodynamic response should be evaluated in a comprehensive manner using appropriate *in vivo* models, taking the sensitivity of the model into consideration.

For instance, changes made to the lipid hydration or blending process or quality grade of the lipid can affect the lipid fluidity or homogeneity of the lipid composition, which consequently has an impact on the drug release and loading efficiency of the active substance. Although these pre- and post-change products appear similar, some differences have been identified in the comparison of quality attributes, and a possible impact on efficacy profiles is not clear. In such situations, the applicant should consider the comparison through pharmacodynamic studies. Furthermore, interactions between the liposome and target cells can affect pharmacodynamics. Therefore, if a change is observed in the following quality attributes that could have an impact on the interactions between the liposome and target cells after the change in manufacturing, the comparison through pharmacodynamic studies should be considered: size, size distribution, morphology (including aggregation), *in vitro* release rate, and modification rate for the liposome drug product whose surface is modified with a ligand (targeting moiety) or antibody.

A1.3 Nonclinical toxicity

In general, further toxicity studies are not needed if similar quality attributes between the pre- and post-change product are confirmed in the quality characterization studies. However, in the following cases, some outcomes of the comparability studies on quality attributes can lead to additional toxicity studies. For instance, if the change in manufacturing introduces new impurities, depending on the impurity type and amount, it might be appropriate to conduct toxicity studies to confirm that there is no adverse impact on safety of the post-change drug product. The change of size, size distribution, morphology (including aggregation), and surface modification of liposome with a ligand (targeting moiety) or antibody caused by the manufacturing changes can affect the tissue and organ distribution. If processes affecting these attributes (e.g., hydration and blending of lipids, and sizing of liposome) are changed, and a detailed comparison of the quality attributes between the pre- and post-change liposome drug

products has shown some similarity (but possible adverse impact on safety and efficacy profiles cannot be excluded), the applicant should consider performing toxicity studies to evaluate the impact of some differences in quality attributes on clinical safety and efficacy.

Infusion reactions by administration of liposome drug products mostly depend on the lipid composition of the liposome. Changes in the manufacturing may not increase the incidence of these reactions as long as the lipid composition remains unchanged. If a change in the manufacturing process or a change in pH or its composition of the aqueous phases outside of the liposome results in an increase in aggregates, size, or size distribution of the liposome, or different loading efficiency, a toxicity study (see Section 4.5) should be considered to evaluate the extent of potential adverse events.[3]

A2 Clinical studies

When quality and nonclinical data are insufficient to establish the comparability of a liposome drug product, additional evidence from clinical studies is required.

A2.1 Clinical pharmacokinetic studies

In principle, cross-over studies designed to appropriately evaluate the comparability of the pharmacokinetics between the pre- and post-change liposome drug products should be employed. However, cross-over studies are not appropriate in some cases (e.g., with liposome drug products with a long half-life). In these circumstances, adequate study design should be determined in consideration of the properties of the liposome drug product. The selection of subjects (i.e., healthy volunteers or patients) should be determined depending on the properties of the liposome drug product and target indications. In general, a single-dose study should be conducted. However, if a multiple-dose administration will provide a suitable evaluation, multiple-dose pharmacokinetic studies should also be considered. Dose levels should be determined based on the pharmacokinetic properties of the product within the proposed clinical dose level, and should be scientifically sound. In principle, blood samples should be collected. The unencapsulated active substance and total active substance (and depending on the properties of the liposome drug product, the encapsulated active substances) should be quantified using validated bioanalytical methods.

The comparability of the pharmacokinetics of an unencapsulated active substance and total active substance should be determined between the pre- and post-change liposome drug product. The primary pharmacokinetic parameters are, for example, Cmax and AUC. Studies using other parameters might be required depending on the pharmacokinetic properties of the liposome drug product. The comparability limits for the pharmacokinetic parameters should be

[3] Szebeni J. Eur J Nanomed. 2012;4:33–53.

defined prior to conducting the study and scientifically justified taking into consideration the effects on the pharmacokinetics, efficacy, and safety of the liposome drug product.

A2.2 Other clinical studies

If data from quality, nonclinical, and clinical pharmacokinetic studies have failed to demonstrate the comparability between drug products before and after the change in manufacturing, additional clinical studies are required (i.e., pharmacodynamic studies indicated by pharmacological effects supporting therapeutic efficacy or clinical efficacy studies indicated by the therapeutic effectiveness in indications).

A2.3 Safety issues

Acute infusion reactions are relatively common following the administration of liposome drug products. If there is any concern that the change in manufacturing would increase the risk of such reactions, the cause should be identified and, where necessary, the formulation development should be also reviewed. Not limited to acute infusion reactions, the safety of liposome drug products has to be compared based on the limited nonclinical and clinical data. Therefore, it is important to continue risk management efforts where necessary, even after marketing has begun.