参考（リフレクションペーパー英訳）

Reflection paper on nucleic acids (siRNA)-loaded nanotechnology-based drug products

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1. Introduction

Currently, various nucleic acid-based compounds are being developed as medicines. Small interfering RNAs (siRNAs) comprise a class of double-stranded RNA molecules, with 21 to 23 base pairs, which enable sequence-specific gene silencing by promoting specific degradation of target mRNAs. Due to their potent mRNA degradation activity and sequence-specificity, siRNAs have been considered potential candidates of pharmaceutical application for over 10 years. In contrast to low-molecular-weight chemical entities, siRNAs have a high molecular weight, are negatively charged, and are highly hydrophilic. These physicochemical properties make the efficient delivery of siRNAs to target cells difficult, which poses a challenge to their pharmaceutical development. In addition, when injected into the blood by itself, siRNA is readily eliminated from the blood by rapid degradation and glomerular filtration through the kidneys, because of its hydrophilic nature and relatively low molecular weight (less than 20 kDa); these properties are also considered major barriers to its pharmaceutical application. To overcome these challenges, many novel delivery techniques such as nanotechnology-based carriers (e.g., liposomes and polymeric micelles) are under development.

Electrostatic interactions with a positively charged carrier or covalent binding are used to bind siRNAs to a carrier. Moreover, lipids and polymers are used in some formulations to improve their pharmacokinetic behavior. In most cases, siRNAs are encapsulated in carriers, while others are not. Most siRNA carriers are incorporated into the cells in the form of nanoparticles through endocytosis. For subsequent transport of siRNAs into the cytoplasm, attempts have been made to engineer the drug product to have an endosomal release mechanism. This must take place before fusion with the lysosome in order to avoid lysosomal degradation. Other attempts have included the introduction of chemically modified nucleic acids with enhanced pharmacodynamic properties and *in vivo* stability. [1, 2]

This reflection paper addresses some points for consideration when assessing siRNA drug products made using nanotechnology-based carriers (hereinafter referred to as nucleic acids [siRNA]-loaded nanotechnology-based drug product).

2. Scope

While this reflection paper discusses the points to be considered for pharmaceutical development of nucleic acids (siRNA)-loaded nanotechnology-based drug product, the principles outlined here may be applicable to the pharmaceutical development of nucleic acids other than siRNA-based products that use nanotechnology-based carriers. With regard to carrier-specific considerations, the relevant notifications and guidelines [3] should be consulted.

The studies and evaluations required for each drug product should be determined on a

case-by-case basis, justified by a reasonable rationale that reflects the current academic and technological advances and the manufacturer's accumulated experience.

3. Quality considerations

The aims of loading siRNAs on a nanotechnology-based carrier are to improve the *in vivo* stability of the siRNA, to deliver the siRNA to the target organ and/or tissue, and, in some cases, to control the intracellular behavior of the siRNA. As carrier components have various functions, the quality of each component may affect the overall quality of the drug product. Therefore, information on the quality of the components should be provided at the same level of detail as expected for the active substances. Furthermore, it is important not only to identify the critical quality attributes that would affect the safety and efficacy of the drug product, (particularly the *in vivo* pharmacokinetic and pharmacodynamic properties) but also to establish test methods for evaluating these quality attributes. If the pharmacokinetic properties are expected to vary (e.g., in the case of change in the carrier components), detailed evaluation of quality attributes, as well as nonclinical evaluation, should be performed again after the change has been made, because the pharmacokinetics of the siRNA depend on the carrier as mentioned above.

3-1 Quality considerations related to pharmacokinetic behavior and delivery to target cells

3-1-1 Considerations related to pharmacokinetic behavior

When nanotechnology-based carriers are used as a means of delivery, it is important to control particle size and surface properties made via modification with, for example, polyethylene glycol (PEG), both of which can affect the retention of siRNAs in the blood because enhanced blood retention would generally affect its delivery to the target organs and/or tissues. In general, the carrier is likely to interact with biological components, and thus, attention should be paid toward the change in stability of the drug product caused by the substitution of the siRNA with a biological component, and with subsequent enzymatic degradation of the siRNA. Loading efficiency of siRNAs onto a carrier can be evaluated by methods such as gel electrophoresis, fluorescent labeling, or intercalation using fluorescent dye. In addition, to ensure that the drug product has a consistent *in vivo* stability and siRNA release profile, *in vitro* test methods using a test solution that appropriately reflects physiological conditions should be established to evaluate the release of siRNA from the carrier.

Certain products are designed to deliver siRNAs to the target organ, tissue, or cells by active targeting delivery using functional molecules (e.g., a ligand [targeting moiety] or an antibody) conjugated to a carrier. In that case, the conjugation of the functional molecule to the carrier

should be optimized so that the linker does not affect the function of the functional molecule. In addition, it should be noted that the properties of the functional molecule or linker could affect the overall properties of the carrier. Therefore, the stability of the linker is also important.

3-1-2 Considerations related to delivery to target cells

To ensure efficient delivery of the siRNA to the target cells, it is important to control the quality attributes and the pharmacokinetic influence of the carrier.

Techniques to enhance intracellular uptake include control of particle size as well as surface properties, including the addition of positive charge to the siRNA carrier, enhancement of cellular membrane fusion, and conjugation of functional molecules.

3-2 Quality considerations related to safety

To reduce the risk derived from carrier-related safety issues, the carrier components and the properties of drug product should be optimized. Examples to be specifically considered are described below: [4]

3-2-1 Optimization of carrier components

- Enhancement of biodegradability
- Use of components with known safety profiles
- Design and optimization of cationic lipids and/or polymers

3-2-2 Optimization of properties of the drug products

- Optimization of particle size
- Masking of the positive charge
- PEGylation of the carrier to improve retention of siRNA in the blood and consequent distribution to the target organ and/or tissue
- Improvement of stability of siRNA-loaded carrier
- Optimization of the product properties relevant to the intended route of administration

3-2-3 Targeting delivery

- Targeting with a ligand molecule (e.g., epidermal growth factor receptor [EGFR], folate receptor, or transferrin receptor)
- Use of cell membrane-permeable peptides etc.

4. Nonclinical considerations

4-1 Nonclinical pharmacokinetic study

To appropriately evaluate the efficacy and safety of siRNAs which are generally delivered with a carrier, it is important to quantify the blood concentration and organ and/or tissue

distribution using the drug product.

- Available assay methods include fluorescent labeling, radioisotope labeling, polymerase chain reaction (PCR), or mass spectrometry. The appropriate assay should be chosen based on the characteristics of the labeling method or sensitivity of the analytical technique.
- The concentrations of unloaded siRNA, siRNA loaded on the carrier, and total siRNA should be measured. Depending on the stability of siRNA in the blood, it may be difficult to measure concentrations of unloaded siRNA (e.g., in case of natural siRNAs).

4-2 Nonclinical toxicity study

In principle, toxicity studies of nucleic acids (siRNA)-loaded nanotechnology-based drug products should be equivalent to those of low-molecular-weight chemical compounds. More specifically, the effects on the target organ and the toxicity effects of nucleic acids (siRNA)-loaded nanotechnology-based drug products, such as *in vivo* cytokine production, should be evaluated. The selection of animal species and study design should be based on the specific characteristics of the properties of the siRNA or the carrier. When released from the carrier, siRNAs chemically modified to improve *in vivo* stability are likely to accumulate in the kidney, which raises toxicity concerns. Therefore, toxicity studies of siRNA alone should be conducted where necessary.

Furthermore, the following factors should be considered regarding siRNAs and their carriers:

4-2-1 Toxicity of the siRNA [5]

To improve the pharmacokinetic behavior of siRNAs, attempts have been made to modify the siRNA chemically and/or to use the carrier to improve both its *in vivo* stability and enhance its targeting delivery. On the other hand, it must be noted that prolonged retention of siRNA in blood and organs and/or tissues could increase siRNA-associated toxicity, and toxicological concerns of interactions between the carrier and the biological components. In general, the following types of toxicity should be considered.

- Immunotoxicity: Activation of the immune system mediated by certain types of Toll-like receptors (TLRs), complement activation, and variations in immune cells
- Hematotoxicity: Hemolysis, blood coagulation, and platelet aggregation

In addition, siRNAs may cause other types of toxicity:

- Toxicity caused by their action on the target sequence
- Toxicity caused by their action on non-target sequences

Appropriate test methods to evaluate these toxicities should be employed.

4-2-2 Toxicity of the carrier [4]

Safety issues relevant to carriers, such as the toxicity derived from interactions between the carrier and biological components, should be addressed.[1] In addition, accumulation following multiple doses and/or long-term administration may raise safety issues.

5. Considerations for first-in-human studies

For first-in-human studies of nucleic acids (siRNA)-loaded nanotechnology-based drug products, the principles described in "Section 3.3 Considerations for first-in-human studies" in the PFSB/ELD Notification No. 0110-1 "Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products" (dated January 10, 2014) should be considered.

References

[1] Castanotto D, Rossi JJ. Nature. 2009;457:426-433.

[2] Cabral H, Kataoka K. J Control Release. 2014;190:465‐476.

[3] Joint MHLW/EMA reflection paper on the development of block copolymer micelle medicinal products, January 10, 2014, PFSB/ELD Notification No.0110-1

[4] Xue HY, Liu S, Wong HL. Nanomedicine (Lond). 2014;9:295-312.

[5] Miyakawa S. J Clin Exp Med. (in Japanese). 2011;238:519-523.

[1] The following cases have been reported as issues relevant to carriers

1) Inflammatory reactions and cytotoxicity caused by interactions of cationic carrier components with plasma membranes and organelles, or inflammatory reactions and cytotoxicity caused by oxygen radical production induced by the carrier components (Ballarín-González B, Howard KA. Adv Drug Deliv Rev. 2012;64:1717-1729., Akhtar S. Expert Opin Drug Metab Toxicol. 2010;6:1347-1362., Lappalainen K, Jääskeläinen I, Syrjänen K, Urtti A, Syrjänen S. Pharm Res. 1994;11:1127-1131., Lv H, Zhang S, Wang B, Cui S, Yan J. J Control Release. 2006;114:100-109.)
2) Effects on gene expression caused by specific dendrimers (Akhtar S, Benter I. Adv Drug Deliv Rev. 2007;59:164-182., Omidi Y, Hollins AJ, Drayton RM, Akhtar S. J Drug Target. 2005;13:431-443.)
3) Genotoxicity caused by specific carbon nanotubes (Tsukahara T, Haniu H. Mol Cell Biochem. 2011;352:57-63.)
4) Immunotoxicity: hematotoxicity, hypersensitivity reactions, and inflammatory reactions etc. (Zolnik BS, González-Fernández A, Sadrieh N, Dobrovolskaia MA. Endocrinology. 2010;151:458-465.)