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Guideline

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Guideline on safety evaluation of cell-based medicinal products for animal use

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ABSTRACT

With the increased use of cell therapy in the veterinary sector, there is a growing demand for the development of cell-based medicinal products and the determination of their safety. Currently, the Korean Animal and Plant Quarantine Agency has established a guideline for evaluating the safety of cell-based medicinal products for animal use. The guideline includes items related to definition, classification, management, manufacturing procedure and quality control (standard and test method), stability testing, toxicity testing, pharmacological testing, and performance of clinical trials. In addition, testing protocols related to safety assessment of animal cell-based products such as chromosome karyotyping, tumorigenicity testing, confirmatory testing of biodistribution and kinetics, and target animal safety testing are described in detail. Moreover, because cell-based medicinal products are novel therapies, deviations from traditional designs may be justified in order to obtain relevant safety information on the treatment. Additionally, this guideline can be amended on the basis of new scientific findings.

Keywords: Cell-based medicinal product; cell therapy; safety evaluation; animal use

This guideline aims to contribute to the proper evaluation of cell-based medicinal products (CBMP) for animal use and to ensure their safety, by presenting standard procedures for the safe evaluation of cell-based medicinal products conducted for manufacturing applications and marketing authorization of new veterinary medicinal products. Details not prescribed in this guideline are to be tested under the approval of the Animal and Plant Quarantine Agency (APQA).

OVERVIEW

Definition

"Cell-based medicinal products" refer to veterinary medicinal products that are manufactured by physical, chemical, and/or biological manipulation of autologous,

allogeneic, and xenogeneic living cells *in vitro*, such as by culturing, multiplying, or selecting [1]. However, cases in which a veterinarian manipulates autologous or allogeneic cells during surgery or treatment in a medical institution, such as an animal hospital, shall be excluded from this category [1].

Classification

Cell-based medicinal products are divided into those derived from animal somatic cells or animal stem cells; furthermore, animal stem cell-derived products can be classified into products from animal adult stem cells, embryonic stem cells, or induced pluripotent stem cells [2,3].

Management

Cell-based medicinal products are managed as general veterinary drugs in terms of their efficacy, while they are managed according to regulations for biologics in terms of their properties and quality [1,3].

MANUFACTURING METHOD STANDARDS AND TEST METHODS

The safety of CBMP should be ensured primarily through quality control according to the manufacturing method of the corresponding product, its manufacturing standards, and the associated test method. Therefore, the following should be documented in accordance with the relevant provisions of CBMP contained in the Regulation on Safety and Efficacy Evaluation for Veterinary Medicinal Products (APQA Notice No. 2018-5) [1].

- Manufacturing process related to cell origin, cell collection (extraction and selection), freezing, thawing, primary culture, secondary culture and filling of cells, and its quality control requirements (storage conditions and periods, etc.)
- Specific screening information on the cell donor animals (whether the animals had infectious diseases, genetic mutations, etc.)
- Evidence of the absence of bacteria, fungi, mycoplasma, endotoxins, or adventitious viruses throughout the manufacturing process

STABILITY TESTING

Stability tests should be conducted according to the testing standard for long-term storage presented in the Guideline on Stability Testing for Veterinary Medicinal Products (APQA Notice). However, as it can be difficult to apply the general stability testing protocol due to the characteristics of CBMP, stability testing may be carried out by an appropriate test method that considers the characteristics of each product. In such a case, the storage method and expiration date shall be established by confirming the product's cell viability and potency through an appropriate stability test that considers the formulation, storage conditions, storage period, and transportation container and transport procedures (including temperature management) of the product, and the test's validity shall be documented. In particular, when a product is frozen or thawed, it shall be confirmed whether such product manipulations influence the stability or specifications of the product.

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TOXICITY TESTING

Toxicity tests shall be conducted in compliance with the Guideline on Toxicity Testing for Veterinary Medicinal Products (APQA Notice No. 2016-22) [4]. However, as it can be difficult to apply the general toxicity testing protocol due to the characteristics of CBMP, toxicity testing may be carried out by using an appropriate test method that considers relevant regulations and the characteristics of each product. Furthermore, unlike general veterinary drugs, a combination of pharmacological and toxicity testing may be considered for CBMP.

Acute, subacute, and chronic toxicity testing

Acute, subacute, or chronic toxicity tests shall be conducted on at least one species of test animal (mouse, *etc.*) after considering the characteristics of the cell-based medicinal product. In such a case, the test may be carried out using a target animal or an appropriate animal model of the disease of interest. The test methods (administration route, dose level, frequency of administrations, observation period, *etc.*) shall be determined based on a comprehensive review of the clinical application method and the biodistribution and kinetics of the corresponding product, while evaluation items shall be determined in a manner that enables the prediction of adverse events, such as toxicity or ectopic tissue formation. Although the specific test method shall follow the relevant test protocol of the Guideline on Toxicity Testing for Veterinary Medicinal Products, that protocol may be followed by the test protocol for safety testing for the target animal.

Reproduction toxicity testing

When, during acute, subacute or chronic toxicity tests of a cell-based medicinal product, the corresponding cells are observed to be present in the gonads or reproductive tissues, or abnormalities in the reproductive system are confirmed, a reproductive toxicity test shall be conducted. The specific test methods should follow the relevant protocols of the Guideline on Toxicity Testing for Veterinary Medicinal Products.

Mutagenicity (or genotoxicity) testing

If a product has the potential to affect DNA or chromosomes, a mutagenicity test shall be conducted. However, the mutagenicity tests used for general veterinary drugs can be meaningless when applied for CBMP due to their characteristics. Therefore, mutagenicity testing can be conducted by confirming the transformation of cells other than the intended targets or by using a chromosome karyotyping test (**Appendix 1**) [5].

Carcinogenicity (or tumorigenicity) testing

When a cell-based medicinal product has tumorigenic potential, such as a product derived from animal stem cells or cells confirmed to have abnormalities in a mutagenicity test, a tumorigenicity test shall be conducted using an immunodeficient animal or an equivalent animal, with the test having an observation period (12 weeks or longer) that is suitable for tumor formation in the target animal body (**Appendix 2**) [5].

Local toxicity testing (or local tolerance testing)

A local toxicity test is conducted for clinical and histopathological evaluation at the injection site where a cell-based medicinal product is administered. This test may be conducted as an evaluation item within an acute, subacute, or chronic toxicity test.

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Immunotoxicity testing (or confirmatory testing of immune system alteration)

When a cell-based medicinal product (limited to autologous cells) has the potential to cause an alteration in the immune system (*e.g.*, changes in hematologic parameters, immunoglobulin levels, organ weights related to the immune system, *etc.*), an immunotoxicity test shall be conducted. Specific test methods shall follow the relevant test protocols of the Guideline on Toxicity Testing for Veterinary Medicinal Products. When there is no appropriate animal model, *in vitro* or *ex vivo* assays may be considered. Finally, this test needs to be included as an item in the evaluation of safety testing for the target animal.

Other special toxicity testing

A special toxicity test that is recognized as necessary according to the characteristics of the specific cell-based medicinal product shall be conducted.

PHARMACOLOGICAL ACTION TESTING

Pharmacological information for estimating the mechanism of action and efficacy of CBMP can be collected, but the data can only be used when the effectiveness of the corresponding products has been reasonably revealed by a review of domestic and foreign literature or knowledge. Furthermore, when a pharmacological action test of a cell-based medicinal product is conducted, it can be combined with toxicity testing; in such a case, the test methods and evaluation criteria should be scientifically and rationally validated.

Efficacy testing

In principle, efficacy testing shall be conducted using an appropriate test animal that can support the mechanism of pharmacological action and the effectiveness of the cell-based medicinal product. The efficacy test should demonstrate the expression, persistence, and effects of the product's pharmacological action. The efficacy test may be conducted as an additional item in the pharmacological action test when using a target animal or an appropriate animal model of the disease of interest.

General pharmacological testing

When a cell-based medicinal product or cell-derived bioactive substances other than the intended target are predicted to have adverse effects on the central nervous system, cardiovascular system, or respiratory system, a general pharmacological test shall be conducted to determine the effects on each system and function.

Testing for absorption, distribution, metabolism, and excretion

A confirmatory test of the biodistribution and kinetics, such as engraftment, proliferation, and persistence in multiple tissues, of a cell-based medicinal product shall be conducted using a target animal or an appropriate animal model of the disease of interest. In particular, when a cell-based medicinal product acts in a specific area (tissue type, *etc.*), its area or tissue localization should be demonstrated [2]. The test methods and evaluation criteria should be scientifically and rationally validated (**Appendix 3**).

CLINICAL TRIALS (INCLUDING TARGET ANIMAL SAFETY TESTING)

In principle, a clinical trial shall be conducted using a target animal or an appropriate animal model of the disease of interest (considering efficacy, compatibility, immune compatibility, *etc.*) only when it is unavoidable in accordance with the Guideline on Clinical Trial Management for Veterinary Medicinal Products (APQA Notice No. 2015-29) [6]. It is recommended that clinical trials should be conducted according to the approved protocol in order to obtain test results that are suitable for verifying the efficacy of the cell-based medicinal product and assessing its safety. When safety testing in a target animal is conducted, an appropriate test plan and evaluation items should be determined after considering the characteristics of each product and the guideline on target animal safety for veterinary pharmaceutical products excluding veterinary biologics (VICH GL43) of the Veterinary International Cooperation on Harmonization (VICH) [3,7,8]. **Appendix 4** contains information on the standard test methods.

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This guideline describes the positions of the Animal and Plant Quarantine Agency (APQA) regarding the safety evaluation of cell-based medicinal products for veterinary use while ensuring scientific objectivity and transparency through expert reviews and inquiries of related associations and industry. Please note that the contents herein should be viewed as recommendations that are not legally enforceable and can be amended when new scientific evidence is presented.

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Appendix 1. Chromosomal Karyotyping Test

1. Overview

The mutagenicity tests used for general veterinary drugs can be meaningless for CBMP due to their characteristics. Rather, as the genomic stability of the cells constituting CBMP may be highly correlated with the tumorigenicity of the products, it is necessary to evaluate their mutagenicity. Therefore, the following presents standard protocols for chromosomal karyotyping tests that can be used to assess the genomic stability of CBMP.

2. Test methods (G-banding or Giemsa banding karyotyping)

2.1. Preparation of cells

For chromosomal karyotype analysis, the cells that constitute the CBMP should be sub-cultured in tissue culture flasks at 37°C with 5% CO_2 and air. Assaying should be conducted when the cultures attain 50% to70% confluence.

2.2. Harvest of cells in metaphase of mitosis

① When cells reach the appropriate confluence, add colcemid (Gibco 15212-012 or equivalent, final concentration 0.1 μg/mL) to the flasks and mix them well. Cultivate the cells at 37°C for 40–50 min. If the treatment time of colcemid is longer, chromosomes shorten and thicken; therefore, the treatment time may need to be adjusted according to the chromosome shape.

② Suspend the cultured cells from flasks, move them to a 15 mL centrifuge tube and centrifuge at 200 × *g* (1,100 r/min) for 5 min.

③ Remove the supernatant from the centrifuge tube, and gradually add 12 mL of pre-warmed (37°C) 0.075M KCl solution dropwise, by gently tapping the side of the centrifuge tube or by slowly mixing with a mixer. Place the tube in a water bath at a constant temperature of 37°C within 20 min. Add 1 mL of Carnoy's fixative solution (3:1 mixture of methanol and glacial acetic acid), mix, and centrifuge at 200 × *g* (1,100 r/min) for 5 min.

④ Again, remove the supernatant from the centrifuge tube and gradually add 7 mL of fixative solution dropwise with gentle tapping, ensuring that each drop runs down the side of the centrifuge tube. (Note: This is the most important step and determines the state of the metaphase of mitosis). Leave the centrifuge tube at room temperature (while maintaining humidity at xx) for 20 min. Then, fill the tube with up to 10 mL of the fixative and centrifuge at 200 × *g* (1,100 r/min) for 5 min.

⑤ Again, remove the supernatant from the centrifuge tube (cell mass and other various residues attached on the wall of the centrifuge tube should be removed carefully using a cotton swab) and suspend the cell layer again with 12 mL of fresh fixative solution. If needed, repeat the washing process with the fixative solution.

2.3. Fabrication of slide samples

It is recommended that slide samples should be fabricated on the day of the harvest of cells in mitosis. The concentration of the cell suspension should be appropriately adjusted by using a fixative solution (1:3 ratio of cell layer to the fixative solution), and two slide samples should be fabricated.

2.4. Staining

The fabricated slides should be dried at 55°C for 12 h or longer, and then undergo Giemsa staining (4%) or Leishman staining (4%).

• Giemsa Staining (4%)

① Dip the slides in a mixture solution* of 0.125% trypsin/0.9% NaCl for 30 sec.
*Mixture solution of 0.125% trypsin/0.9% NaCl: 100 mL of 0.5% trypsin + 300 mL of 0.9% NaCl

② Stop the trypsin reaction by moving the slides to 0.9% NaCl immediately (repeat steps 1 and 2 twice).

③ Stain the slides with Giemsa staining solution* for 5 min.
*Giemsa staining solution: 485 mL of Gurr's buffer solution (phosphate buffer solution, pH 6.8) + 30 mL of Giemsa solution + 10 mL of acetone

④ After staining the slides, wash them with Gurr's buffer solution (repeat steps 3 and 4 twice).

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3. Detection method

3.1. The number of chromosomes should be counted in 20 or more cells that exhibit mitotic metaphase, and karyotype analysis should be performed on at least 5 cells in mitotic metaphases.

3.2. At least two rounds of karyotype analysis should be performed for each sample, and chromosomal abnormalities should be determined by referencing the International System for Cytogenetic Nomenclature.

Appendix 2. Tumorigenicity Testing

1. Overview

Tumorigenicity testing of CBMP is based on animal experimentation that uses live test animals. In this study, it is important to select animal models that can survive for a sufficient time and that can allow for the development of a prediction of tumorigenic potential in clinical practice. As needed, the test may be conducted additionally using *in vitro* tests, *etc.*

2. Test methods

2.1. Test animal

Appropriate animals, for which CBMP can engraft in vivo and exhibit pharmacological activity, should be selected and should include both male and female animals. In order to prevent test animals from having an immune response to the product, it may be necessary to select animals with impaired immunity or immunodeficiency or to consider the administration of immunosuppressive agents.

2.2. Number of animals

The number of animals should be determined after considering the sensitivity of test animals, the test methods, and the precision of the examination items. At this time, the loss of animals due to sudden death during the test and the number of animals required for the evaluation of tumorigenesis should also be taken into consideration, and finally, the number of animals that enable interpretation of the tumorigenicity results should be determined. In general, more than ten animals of each sex per group shall be used.

2.3. Route of administration

In principle, the administration route that is applied in clinical practice should be used. At this time, it is important to administer CBMP to the test animals through the same route as that in clinical applications and to ensure that the products are engrafted and distributed within the same anatomical area. When the administration route is different from that used in clinical applications, the route deemed most similar should be selected, and the scientific basis, including reasons and feasibility, for the selected route should be presented.

2.4. Dose level

Test group and control group are to be tested. The test group should consist of three or more dose levels, with the highest recommended dose in clinical application being the lowest level. The highest dose should be determined as the maximum feasible dose (generally, 107) that can be administered to test animals. It is desirable for the middle dose to be the geometric mean of the highest and lowest doses.

2.5. Control group

The control groups consist of both negative and positive groups. For the positive control group, the tumor-forming cell lines should be selected from cell lines that are similar to the CBMP; whereas, for the negative control group, an appropriate form of solvent (excipient, *etc.*) should be used.

2.6. Frequency and duration of administration

The frequency and maximum duration of administration should be selected based on the product's intended use in clinical practice.

2.7. Duration of study

An observation period appropriate for the study should be provided after the end of administration. A sufficient test period (12 weeks or longer) should be ensured, as CBMP may vary in the speed or frequency of tumorigenesis depending on the engraftment period of the product in the selected test animals due to their characteristics.

3. Detection method

3.1. The general health and behavior (including food and water consumption) of all animals in each group shall be observed daily and in detail. Body weights shall be measured at regular intervals after the beginning of administration.

3.2. In order to evaluate the tumorigenesis process, a quantitative visual examination (size, etc.) should be conducted at regular intervals, if grossly observable. Although the observation of tumors should be performed at the end of the study, it is also recommended to conduct an evaluation related to tumorigenesis during the test, such as assessment of the engraftment pattern of cells and the presence of reversible lesions (usually hyperplasia) in some animals.

3.3. For animals that are dead or facing imminent death during the study, necropsy should be performed immediately. Gross and histopathological examinations of organs and tissues (skin, mammary gland, lymph nodes, salivary glands, sternum, vertebra or femur [including bone marrow], thymus, trachea/lung and bronchi, heart, thyroid and parathyroid, tongue, esophagus, stomach and duodenum, small intestine, large intestine, liver, pancreas, spleen, kidney, adrenal gland, bladder, seminal vesicle, prostate, testes, ovary, uterus, vagina, eyeball, brain, hypophysis, spinal cord, and other organs and tissues with grossly tumorous lesions) should be conducted. If the animals that are dead or facing imminent death during the study are judged to be in that condition due to factors that are irrelevant to the test substance, a histopathological examination may be conducted only on the major organs. Furthermore, if it is possible to collect blood samples, it is desirable to conduct hematology and blood chemistry.

3.4. For all animals that survive to the end of the study, necropsy should be done promptly, and gross and histopathological examinations of the same organs and tissues as specified in 3.3 should be conducted. As needed, hematology and blood chemistry, etc. may also be required.

Appendix 3. Confirmatory Test of Biodistribution and Kinetics

1. Overview

It is difficult to apply results of studies into the absorption, distribution, metabolism, and excretion of general veterinary drugs to cell-based medicinal products (CBMP) due to their specific characteristics. Therefore, it is reasonable to conduct tests that confirm aspects of biodistribution and kinetics, such as engraftment, proliferation (or differentiation), and persistence of CBMP in tissues by using a target animal or an appropriate animal model of the disease of interest. To conduct such a test, quantitative polymerase chain reaction (PCR) amplification, immunochemical analysis, and cellular imaging techniques (radioisotope labeling, genetic transformation including luciferase expression, *etc.*) can be utilized. When cells are transformed to use a cellular imaging technique, it should be determined that such modification does not affect the survival and function of the cell-based medicinal product.

2. Test methods

2.1. Test animal

After considering the genetic relationships of animals with the CBMP, appropriate animal models, in which the products can engraft in vivo and exhibit pharmacological activity similar to that in clinical applications, should be selected. In order to prevent test animals from having an immune response to the product, it may be necessary to select animals with impaired immunity or immunodeficiency or to consider the administration of immunosuppressive agents.

2.2. Number of animals

The number of animals should be determined after considering the sensitivity of the test animals, the test methods, and the precision of examination items. As CBMP may proliferate or differentiate during the test, a sufficient number of animals (including males and females) should be tested to allow for the results to be suitably interpreted in light of this possibility.

2.3. Route of administration

In principle, the administration route that would be applied in clinical practice should be used. At this time, it is important to administer CBMP to test animals through the same route as that in clinical applications, and to ensure that the products are engrafted and distributed in anatomically similar areas. When the administration route is different from that used in clinical applications, the route that is deemed most similar should be selected, and the scientific basis for selecting the route, such as the reason for and feasibility of use, should be presented.

2.4. Dose level

A test group and control group are to be tested. The test group should consist of a minimum of two dose levels, with the highest recommended dose in clinical application being the lowest level. The test group shall include a multiple (generally three to ten times) of the highest recommended clinical dose, and it may be necessary to set up test groups separately for single dose and repeated dose testing when repeated dosing is used in clinical applications.

2.5. Frequency and duration of administration

The frequency and maximum duration of administration should be selected based on the intended use in clinical practice.

2.6. Duration of study

An observation period appropriate for the study should be provided after the end of product administration. Depending on the characteristics of the cell-based medicinal product, a sufficient test period for engraftment and processing in the test animals should be ensured.

3. Detection method

3.1. The general health and behavior of all animals in each group shall be observed daily and in detail. It is recommended that a necropsy is performed immediately for animals that die or are faced with imminent death during the study, and to conduct both gross and histopathological examinations.

3.2. In principle, the biodistribution and kinetics of a cell-based medicinal product should be confirmed to assess the distribution pattern and persistence time at the application sites and in the target organs of the product. Evaluation of the product's distribution pattern in other major organs (brain, lung, liver, heart, spleen, testicles/ovary, kidney, pancreas, bone marrow, blood, lymph nodes, etc.) is recommended. When evidence that CBMP are to be applied locally and that they do not have the potential for systemic exposure have been presented, the evaluation can be conducted only at the application sites and in the target organs of the CBMP, as well as in neighboring tissues.

3.3. When quantitative PCR is conducted, it is preferable to detect and quantify specific DNA sequences according to the autologous, allogeneic, and xenogeneic relationship between the cell-based medicinal product and the test animals. The validation data (linearity, accuracy, precision, specificity, limit of detection [LOD], limit of quantification [LOQ], etc.) of the test methods may be required.

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Appendix 4. Target Animal Safety Testing

1. Overview

Currently, a guideline on target animal safety testing for veterinary medicinal products has not been established. Therefore, in this appendix are presented standard protocols based on the guideline on target animal safety for veterinary pharmaceutical products (excluding biologicals) (VICH GL43) of the Veterinary International Cooperation on Harmonization (VICH) to ensure the safety of cell-based medicinal products.

2. Test methods

2.1. Test animal

Animals that, in terms of age, sex, pregnancy, *etc.*, can represent target animals to which the cell-based medicinal product will be applied shall be employed. Healthy animals should be used, and their feeding and medication histories and breeding method prior to the beginning of the study should be available. If there is no gender restriction in the application of the cell-based medicinal product, both sexes should be included.

2.2. Number of animals

Based on veterinary, animal welfare, and statistical considerations, the minimum possible number of animals shall be used. Generally, more than two animals of each sex per group shall be used.

2.3. Route of administration

In principle, the administration route applied in clinical practice should be used for cell-based medicinal product testing. When multiple routes of administration are proposed in the clinical application of the product, the route that is most likely to cause adverse effects shall be selected.

2.4. Dose level

A test group and control group are to be tested. The test group should consist of a minimum of two dose levels, with the highest recommended dose in clinical application being the lowest level tested. The test shall include a group to which a multiple (generally three times or ten times) of the highest recommended clinical dose is administered for a period of time in excess of the recommended maximum duration of treatment to ensure an appropriate margin of safety for the product.

2.5. Frequency and duration of administration

The frequency and maximum duration of administration should be selected based on the intended use in clinical practice. However, when a product is applied for a long period in clinical practice, the administration period tested may be shortened after the adverse effects of the product have been identified.

2.6. Duration of study

An observation period appropriate for the studies should be added after the end of product administration.

3. Detection method

3.1. The general health and behavior (including food and water consumption) of all animals in each group shall be observed daily and in detail. Body weights shall be measured at regular intervals after the beginning of product administration. Prior to the study, the potential of an undesirable immune response caused by the products should be reviewed and discussed.

3.2. Hematology and blood chemistry analyses should be conducted on all or some of the tested animals during the study. If appropriate, urinalysis and other clinical physical examinations should also be conducted.

3.3. For animals that are dead or facing imminent death during the study, necropsy should be performed immediately, and gross and histopathological examinations should be conducted. If it is possible to collect blood samples, it is desirable to include hematological and blood chemistry analyses.

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3.4. Hematological and blood chemistry analyses should be conducted on all animals that survive during the study. If needed, and as appropriate, other clinical physical examinations, necropsy, and histopathological examination should be conducted.

3.5. As needed, the potential for the product to affect the test animals, as well as normal cells and tissues, should be reviewed and discussed.

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