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Assessment of biodistribution using mesenchymal stromal cells: Algorithm for study design and challenges in detection methodologies

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Abstract

Background aims. Biodistribution of candidate cell-based therapeutics is a critical safety concern that must be addressed in the preclinical development program. We aimed to design a decision tree based on a series of studies included in actual dossiers approved by competent regulatory authorities, noting that the design, execution and interpretation of pharmacokinetics studies using this type of therapy is not straightforward and presents a challenge for both developers and regulators. *Methods.* Eight studies were evaluated for the definition of a decision tree, in which mesenchymal stromal cells (MSCs) were administered to mouse, rat and sheep models using diverse routes (local or systemic), cell labeling (chemical or genetic) and detection methodologies (polymerase chain reaction [PCR], immunohistochemistry [IHC], fluorescence bioimaging, and magnetic resonance imaging [MRI]). Moreover, labeling and detection methodologies were compared in terms of cost, throughput, speed, sensitivity and specificity. *Results.* A decision tree was defined based on the model chosen: (i) small immunodeficient animals receiving heterologous MSC products for assessing biodistribution and other safety aspects and (ii) large animals receiving homologous labeled products; this contributed to gathering data not only on biodistribution but also on pharmacodynamics. PCR emerged as the most convenient technique despite the loss of spatial information on cell distribution that can be further assessed by IHC. *Discussion.* This work contributes to the standardization in the design of biodistribution studies by improving methods for accurate assessment of safety. The evaluation of different animal models and screening of target organs through a combination of techniques is a cost-effective and timely strategy.

Key Words: *advanced therapy medicines, animal models, biodistribution, mesenchymal stromal cells, preclinical*

Introduction

Among current developments in cell-based medicines, multipotent mesenchymal stromal cells (MSCs) account for numerous clinical trials in the fields of regenerative medicine, immunotherapy and organ transplantation [1]. From a regulatory perspective, cell-based medicinal products (CBMPs) must be manufactured according to current Good Manufacturing Practices, and regulatory authorities evaluate quality and safety data before use in humans [2,3]. However, limited understanding of the complex biology of these products and their behavior in the host pose unprecedented challenges to both regulators and developers for the standardization of methods for cellular characterization and manufacture.

Regarding product quality, the International Society for Cellular Therapy has established a minimal criteria

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for defining the identity and potency of human MSCs [4]. However, batches of MSCs differ in their characteristics as a result of the (i) source tissue (typically, bone marrow, adipose tissue and umbilical cord), (ii) donor variability, and (iii) the existing diversity of procedures for their isolation, expansion, storage and final formulation [5–8].

Even more critical is the standardization of strategies aiming at evaluating preclinical safety of CBMP, which cannot be addressed by following methods commonly used with small molecule drugs or biopharmaceuticals [9,10]. Scarce instructions and poorly described recommendations in current guidelines lead to a case-by-case assessment in agreement with the competent regulatory authority. Moreover, this type of studies are expected to be conducted in accordance with Good Laboratory Practices [11].

Major safety concerns for CBMP include their (i) biodistribution, which involves tracking, homing and persistence; (ii) tumorigenic potential, or the appearance of genetic abnormalities acquired by the cells during the manufacturing process; and (iii) immunogenicity, despite MSCs being immunoprivileged due to low-level expression of HLA-DR. Tracking cells within the recipient is key to a proper evaluation of their therapeutic effect as well as an objective risk assessment with respect to inappropriate ectopic tissue formation, alteration of the microenvironment at the engraftment site, or tumorigenicity [9,10,12].

Biodistribution patterns of MSCs in the host may differ for each route of administration and condition to treat. If cells are to be administered intravenously, rather than locally, broad dissemination is likely to occur [13,14]. Even after local administration, cells can still migrate, extravase and eventually diffuse to other tissues and can potentially alter their phenotype by responding to either physiologic or pathologic microenvironments [14], and this may lead to unwanted effects. Therefore the understanding of cell persistence, distribution and behavior of the cells after administration are key before clinical use.

In the present study, preclinical biodistribution studies supporting current clinical trials of MSC-based products are presented and their advantages critically evaluated. We aimed to define a decision tree based on these data to assist in the design of biodistribution studies and the choice of methodologies for labeling and detection, thus contributing to standardization.

Methods

Animals

All animal care and experimental procedures adhered to the recommendations of local, national and European laws (Decret 214 de 1997, Real Decreto 53 de 2013 and European directive 86/609/CEE of 1986, respectively) and were approved by local Ethical Committees on Human and Animal Experimentation of the Universitat Autònoma de Barcelona's, Institut de Recerca de l'Hospital de la Vall d'Hebron's and the Hospital Clínico Universitario Virgen de la Arrixaca, Universidad de Murcia. Animal species and strains used are listed in Table I.

Cell therapy product preparation

Clinical-grade MSCs derived from either bone marrow (BM) or Wharton's jelly (WJ) were produced within the context of five clinical trials (ClinicalTrials.gov identifiers NCT01227694, NCT01605383, NCT02630836, NCT02566655 and NCT03003364) with appropriate donor-informed consent and were further expanded *in vitro* by using Dulbecco's Modified Eagle's Medium (Gibco) supplemented with 10–20% hSerB (Banc de Sang i Teixits) containing 2 mmol/L glutamine [15,17,18].

Fucosylated BM-MSCs were generated from samples taken from three volunteers (ClinicalTrials.gov identifier NCT02566655) with appropriate donor-informed consent and approval by the local ethics committee. Briefly, cells were seeded in low-glucose α-MEM 94% (Gibco) supplemented with 5% human platelet lysate (hPL; Centro Regional de Hemodonación de Murcia), 50 U/mL penicillin/streptomycin (Lonza Biologics) and 2 U/mL heparin (Mayne Pharma) using TrypLE Select (Gibco) for lifting cells in each culture passage [19]. After the third passage, exo-fucosylation was performed using α-(1,3)-fucosyltransferases VI (FTVI) (R&D Systems) and 1 mmol/L GDP-fucose as substrate (Sigma-Aldrich). Reactions were performed at 37°C for 1 h with gentle shaking followed by centrifugation and washing with Dulbecco's phosphate-buffered saline (PBS; Gibco) [20].

Ovine MSCs were derived either from BM aspirates [21] or from amniotic fluid as briefly described next. Ovine amniotic fluid MSCs (oAF-MSC) cultures were started with the isolation of plastic-adherent cells followed by a cell expansion step. In the isolation stage, 300 cm^2 total surface culture area (Corning) were seeded with 0.27 mL oAF per square centimeter in M199 (Gibco, Invitrogen) supplemented with 10% v/v fetal bovine serum (Biological Industries), 5 ng/mL basic Fibroblast Growth Factor (bFGF; Cellgenix) and 1 × penicillin/streptomycin/amphotericin B (Gibco, Invitrogen). Non-adherent cells and debris were removed by washing with PBS (Thermo Fisher Scientific Hyclone) after 3 days of culture and adherent cells cultured with fresh growth medium that was subsequently changed twice a week. After 10 days in culture, cells were harvested using 0.05% trypsin-EDTA dissociation solution (Gibco,