Annals of Medicine and Surgery 24 (2017) 61–64

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.elsevier.com/locate/amsu

Clinical use of adipose-derived stem cells: European legislative issues

Edoardo Raposio[a,b,*], RosaGemma Ciliberti[c]

[a] Department of Medicine and Surgery, Plastic Surgery Section, University of Parma, Italy
[b] Cutaneous, Mini-invasive, Regenerative and Plastic Surgery Unit, Parma University Hospital, Parma, Italy
[c] Department of Sciences of Health (DISSAL), Forensic and Legal Medicine Section, University of Genova, Italy

ARTICLE INFO

Keywords:
Adipose-derived stem cells
Clinical applications
European legislation

ABSTRACT

Objective: With this study we analyse the current European legislation in order to provide guidance for regenerative medicine professionals on correct Adipose-derived Stem Cells (ASCs) isolation and use protocols for clinical applications.

Materials and Methods: The European Medicines Agency (EMA) considers that ASCs does not fall within the definition of an advanced therapy medicinal product if the cells have not been subjected to a substantial manipulation, and the mode of action of the cells (contribute to and enhance tissue renewal and turnover of the subcutaneous tissue) is considered to be homologous to the donor fat tissue.

Results: Collagenase digestion, as well as cell culturing, is considered to be a substantial manipulation. Only transplantation of a non-manipulated tissue to another location in the same anatomical or histological environment is considered to be homologous.

Conclusions: According to these considerations, ASCs should be not-cultured, isolated mechanically and used only in the subcutaneous tissue.

1. Objective

To date, albeit the best characterized adult stem cell population considered to possess multipotent capacity is that of bone marrow Mesenchymal Stem Cells (MSCs), white adipose tissue has been given attention for being the most abundant and accessible source of stem cells in the adult human body [1,2]. By definition, Adipose-derived Stem Cells (ASCs) are plastic-adherent, proliferative, multipotent cells, isolated from adipose tissue and able to exist in an undifferentiated state, also undergoing self-renewal and multilineage differentiation, leading to terminally differentiated cells [3–7]. For these potentials, and since they can be easily harvested in a great amount with minimal donor site morbidity, ASCs proved to be particularly promising for regenerative therapies [8–14].

It is noteworthy though that there is not a standardised protocol to isolate ASCs for clinical application, which led to an inconstancy in literature. To date, there is also a shortage in clinical reports involving ASCs in cell therapy on humans: works available in literature mostly used basic-research-derived protocols and/or other applications than clinical ones [15–28]. Hence, there is need of a standardised method for clinical purposes, which optimize and unify process schedule and isolation procedure, as well as the whole tissue manipulation. Nevertheless, in Europe these protocols have to fall within the strict legislative borders recently set by the European Medicines Agency (EMA). The EMA is a decentralized agency of the European Union (EU), located in London. It began operating in 1995. The Agency is responsible for the scientific evaluation, supervision and safety monitoring of medicines and cellular therapies for use in the EU.

With this study, we analyse the current European legislation in order to provide guidance for regenerative medicine professionals on correct ASCs isolation and use protocols for clinical applications.

2. Materials and Methods

New scientific progress in cellular and molecular biotechnology has led to the development of advanced therapies, such as gene therapy, somatic cell therapy, and tissue engineering. This nascent field of biomedicine offers new opportunities for the treatment of diseases and dysfunctions of the human body. Insofar as advanced therapy products are presented as having properties for treating or preventing diseases in human beings, or that they may be used in or administered to human beings with a view to restoring, correcting or modifying physiological functions by exerting principally a pharmacological, immunological or metabolic action, they are biological medicinal products within the meaning of Annex I to Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community

* Corresponding author. Department of Medicine and Surgery, Plastic Surgery Section, University of Parma, Via Gramsci 14, 43126, Parma, Italy.
E-mail address: edoardo.raposio@unipr.it (E. Raposio).

https://doi.org/10.1016/j.amsu.2017.11.002
Received 26 June 2017; Accepted 7 November 2017

code relating to medicinal products for human use [29],

Further to the implementation of Article 17 of Regulation (EC) No 1394/2007 (the Advanced Therapy Medicinal Products (ATMPs) Regulation), applicants have access to an optional procedure which is the CAT (Committee for Advanced Therapies) scientific recommendation for the classification of ATMPs. The Committee is responsible for assessing the quality, safety and efficacy of advanced therapy medicines, including medicines classified as gene therapy, somatic cell therapy or tissue-engineered products. It is underpinned by the ATMP Regulation which enables the EMA in close collaboration with the European Commission to determine whether or not a given product meets the scientific criteria, which define ATMPs. The ATMP classification procedure has been established in order to address questions of borderline cases where classification of a product based on genes, cells or tissues is not clear. The CAT issues scientific recommendations determining whether or not the referred product falls, within the definition of an ATMP in the European Union. The ATMP Regulation and the Directive 2001/83/EC Annex I Part IV [30] provide precise legal definitions for ATMPs.

The ATMP classification is based on the evaluation of whether a given product fulfils one of the definitions of gene therapy medicinal product (GTMP), somatic cell therapy medicinal product (sCTMP) or tissue engineered product (TEP) and whether the product fulfils the definition of a combined ATMP or not. However, it is also acknowledged that, due to the complex nature of these therapeutic products, the limited data package at an early stage of product development and the rapid evolution of science and technology, questions of borderline may arise.

2.1. Somatic cell therapy medicinal product

Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics:

(a) contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor;
(b) is presented as having properties for, or is used in or administered to human beings with a view to treating, preventing or diagnosing a disease through the pharmacological, immunological or metabolic action of its cells or tissues.

For the purposes of point (a), the manipulations listed in Annex I to Regulation (EC) No 1394/2007, in particular, shall not be considered as substantial manipulations: cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilization, irradiation, cell separation, concentration or purification, filtering, lyophilization, freezing, cryopreservation, and vitrification.

In order to be considered a somatic cell therapy medicinal product, both the characteristics (a) and (b) have to be fulfilled.

2.2. Tissue engineered products

Tissue engineered products, according to Article 2 of Regulation (EC) No. 1394/2007, means a product that:

- contains or consists of engineered cells or tissues, and
- is presented as having properties for, or is used in or administered to human beings with a view to regenerating, repairing or replacing a human tissue.

A tissue engineered product may contain cells or tissues of human or animal origin, or both. The cells or tissues may be viable or non-viable. It may also contain additional substances, such as cellular products, biomolecules, biomaterials, chemical substances, scaffolds or matrices. Products containing or consisting exclusively of non-viable human or animal cells and/or tissues, which do not contain any viable cells or tissues and which do not act principally by pharmacological, immunological or metabolic action, are excluded from this definition.

To be considered ‘engineered’, cells or tissue(s) should fulfil at least one of the following conditions:

2.2.1. Substantial manipulation

The cells or tissue(s) have been manipulated during the manufacturing process so that their biological characteristics, physiological functions or structural properties have been modified to be relevant for their intended function. Examples of substantial manipulations include cell expansion (culture), genetic modification of cells, and differentiation/activation with growth factors.

Cell culturing leading to expansion is considered substantial manipulation. Induction of proliferation of cells during cell culture has to be regarded as changes of their biological characteristics and structural properties, either because of an immediate change in cell functionality or cell phenotype, or by increasing cell numbers to augment the desired function of the cells. Furthermore, most adherent cells, for example, are impacted by the repeated attachment and detachment cycles. It has been demonstrated that even the techniques applied for cell detachment might lead to different phenotypic changes especially on cell surface proteins (e.g. membrane receptors).

Enzymatic digestion of a tissue to release cells is also considered to be substantial manipulation, when the aim is to dissociate cell-cell contacts and the released cells are administered into patients with or without subsequent manipulation. An example would be keratinocytes from skin, for which enzymatic digestion would destroy the tissue architecture and functional interactions of the cells, which cannot be regained in the cell suspension: this would be considered as substantial manipulation.

If the enzymatic digestion leads to isolation of functionally intact tissue units (e.g. pancreatic islets) or there is scientific evidence that the original structural and functional characteristics are maintained, the procedure is not considered substantial manipulation.

In case a tissue is treated to remove cells and to be used without any cellular components (e.g. amniotic membrane, bone) the product is not an ATMP because it does no longer contain cells or tissue. Additionally, based on scientific considerations, the CAT can also consider other manipulations as “non-substantial”. One example is the radiolabeling of leukocytes for diagnostic purposes. This technique has no significant impact on the functional properties of the cells and should thus not be considered a substantial manipulation.

If the number of certain cells (e.g. ASCs in fat grafts) is enriched by selection and the processing does not change the characteristics of the cells, this is not considered a substantial manipulation.

2.2.2. Different essential function (non-homologous use)

In case no substantial manipulation of the cells/tissues takes place, the classification is based on the essential function of the cells/tissues. Such non-substantially manipulated cells or tissues used for the same essential function are not considered ATMPs. The same essential function for a cell population means that the cells when removed from their original environment in the human body are used to maintain the original function(s) in the same anatomical or histological environment. Examples of this category are bone marrow cells or peripheral blood cells used for hematopoietic or immune reconstitution. Other clinical uses of bone marrow cells would be considered to be ATMPs, unless the same essential function(s) and the same anatomical/histological environment can be demonstrated for the cells/tissues both at the donor and administration site (tissue). The same principle applies to other non-substantially manipulated cells from various origins, for example adipose cells transplanted to other than fat tissue are considered to be

ATMPs.

Replacement of a tissue as its whole or functional unit of a tissue (such as cornea or pancreatic islets) is regarded as use for the same essential function. Similarly, transplantation of a non-manipulated tissue to another location in the same anatomical or histological environment is also considered to achieve the same essential function. This is the case for skin transplantation from one part of the body to another part, subcutaneous implantation of pancreatic islets or replacement of arteria by veins. However, in the case of pancreatic islets, the classification will also depend on the manipulation and functional integrity of the islets.

3. Results

The use of enzymatic digestion of the tissue using collagenase that is harmless to cells but rather dissolves collagen fibers is regulated as non-ATMP (pancreatic islets) but is suggested to be regulated as substantial manipulation for other cells, for example ASCs from adipose tissue [31]. Cell populations derived by mechanical purification of tissue do not fall within the definition of a sCTMP. Examples include cryopreserved ASCs or regenerative cells and suspensions of viable, adult, autologous, unexpanded, and uncultured regenerative cells of stromal vascular fraction from subcutaneous adipose tissue (EMA/500724/2012, EMA/129056/2013). In a recent scientific recommendation [32] on classification of advanced therapy medicinal products, the EMA evaluated the clinical use of adult autologous ASCs for subcutaneous administration. In this recommendation, ASCs were described as suspension of viable, adult, autologous, unexpanded, and uncultured regenerative cells of stromal vascular fraction of subcutaneous adipose tissue. The product by itself was indicated for regeneration, repair, or replacement of weakened or injured subcutaneous tissue.

The EMA considered that:

- The product contained viable cells that have not been subjected to a substantial manipulation.
- The mode of action of the product (contribute to and enhance tissue renewal and turnover of the subcutaneous tissue) was considered to be homologous to the donor fat tissue.

Based on the above considerations, the EMA considered that this kind of ASCs did not fall within the definition of an advanced therapy medicinal product.

4. Conclusions

Cell therapies have specific features compared to other medicinal products. The reflection paper is useful and provides an important guide for the interpretation of the Regulation (EC) No 1394/2007. Good surgical practise using manipulated cells as “concurrent treatment” is not defined in EU legislation. Regulation 1394/2007 was established to ensure that patients are not put into undue risk and that products without proven safety and efficacy are not used to treat patients. Substantial manipulation is defined as any processing that alters the original relevant biological, physiological or structural characteristics of cells or tissues. Tissue dissociation to a single cell state usually requires several steps including collagenase treatment (to digest extracellular matrix) and when needed, broad-specificity proteases (e.g. trypsin) to disperse tightly associated cells. These stable cell-cell interactions through gap junctions, tight junctions, adherent junctions and desmosomes play crucial role for the biological activity or structural characteristics of cells or tissues. These types of intercellular interactions are distinguished from those between cells and the extracellular matrix and need specific proteases to cleave them. That being said, we have to keep in mind that recombinant collagenase used to digest extracellular matrix are contaminated with Trypsin-Like-activity due likely to copurification of clostripain which is responsible for most if not all this activity since it is difficult to separate clostripain from collagenase because of its charge heterogeneity. In addition, enzyme-digested tissues might also induce cleavage of a wide variety of cell membrane receptors leading to alteration of cell biological activities. Therefore, CAT can consider that enzymatic digestion will be assessed on a case by case basis and will depends on the nature of the tissue to be digested and deviation may always be possible when scientific evidence is provided. In summary, the use of collagenase for separation of cells from extracellular matrix of tissue is considered a substantial manipulation. Furthermore, ASCs intended to be used in other tissues except for adipose subcutaneous tissue are always considered an advanced therapy medicinal product. According to these considerations, ASCs should be not-cultured, isolated mechanically and used only in the subcutaneous tissue.

Ethical approval

N/A.

Sources of funding

None.

Author contribution

Rosagemma Ciliberti: Study design.
Edoardo Raposio: Data collection and writing.

Conflicts of interest

None.

Guarantor

Edoardo Raposio.

References

[1] E. Raposio, G. Caruana, M. Petrella, S. Bonomini, M.P. Grieco, A standardized method of isolating adipose-derived stem cells for clinical applications, Ann. Plast. Surg. 76 (2016) 124–126.
[2] E. Raposio, G. Caruana, S. Bonomini, G. Libondi, A novel and effective strategy for the isolation of adipose-derived stem cells: minimally manipulated adipose-derived stem cells for more rapid and safe stem cell therapy, Plast. Reconstr. Surg. 133 (2014) 1406–1409.
[3] C. Scanarotti, A.M. Bassi, M. Catalano, C. Guida, R. Coradeghini, C. Falugi, M. Aluigi, P. Santi, E. Raposio, Neurogenic-committed human pre-adipocytes express CYP1A isoforms, Chem. Biol. Interact. 184 (2010) 474–483.
[4] E. Raposio, C. Guida, I. Baldelli, F. Benvenuto, M. Curto, L. Paleari, F. Filippi, R. Fiocca, G. Robello, P.L. Santi, Characterization and induction of human pre-adipocytes, Toxicol. In Vitro 21 (2007) 330–334.
[5] R. Coradeghini, C. Guida, C. Scanarotti, R. Sanguineti, A.M. Bassi, A. Parodi, P.L. Santi, E. Raposio, A comparative study of proliferation and hepatic differentiation of human adipose-derived stem cells, Cells Tissues Organs 191 (2010) 466–477.
[6] M.G. Aluigi, R. Coradeghini, C. Guida, C. Scanarotti, A.M. Bassi, C. Falugi, P. Santi, E. Raposio, Pre-adipocytes commitment to neurogenesis 1: preliminary localisation of cholinergic molecules, Cell Biol. Int. 33 (2009) 594–601.
[7] E. Raposio, C. Guida, R. Coradeghini, C. Scanarotti, A. Parodi, I. Baldelli, R. Fiocca, P.L. Santi, In vitro polydeoxyribonucleotide effects on human pre-adipocytes, Cell Prolif. 41 (2008) 739–754.
[8] G. Caruana, N. Bertozzi, E. Boschi, M. Pio Grieco, E. Grignaffini, E. Raposio, Role of adipose-derived stem cells in chronic cutaneous wound healing, Ann. Ital. Chir. 86 (2015) 1–4.
[9] F. Simonacci, N. Bertozzi, M.P. Grieco, E. Grignaffini, E. Raposio, Autologous fat transplantation for breast reconstruction: a literature review, Ann. Med. Surg. Lond. 12 (2016) 94–100.
[10] J. Fan, E. Raposio, J. Wang, R.E. Nordström, Development of the inframammary fold and ptosis in breast reconstruction with textured tissue expanders, Aesthet. Plast. Surg. 26 (2002) 219–222.
[11] I. Porro, A. Schenone, M. Fato, E. Raposio, E. Molinari, F. Beltrame, An integrated environment for plastic surgery support: building virtual patients, simulating interventions, and supporting intraoperative decisions, Comput. Med. Imaging Graph 29 (2005) 385–394.

[12] E. Raposio, G. Libondi, N. Bertozzi, E. Grignaffini, M.P. Grieco, Effects of topic simvastatin for the treatment of chronic vascular cutaneous ulcers: a pilot study, J. Am. Coll. Clin. Wound Spec. 7 (2016) 13–18.
[13] E. Raposio, S. Bonomini, F. Calderazzi, Isolation of autologous adipose tissue-derived mesenchymal stem cells for bone repair, Orthop. Traumatol. Surg. Res. 102 (2016) 909–912.
[14] E. Raposio, N. Bertozzi, S. Bonomini, G. Bernuzzi, A. Formentini, E. Grignaffini, M. Pio Grieco, Adipose-derived stem cells added to platelet-rich plasma for chronic skin ulcer therapy, Wounds 28 (2016) 126–131.
[15] F. Simonacci, N. Bertozzi, E. Raposio, Off-label use of adipose-derived stem cells, Ann. Med. Surg. 24 (2017) 44–51.
[16] E. Raposio, F. Simonacci, R.E. Perrotta, Adipose-derived stem cells: comparison between two methods of isolation for clinical applications, Ann. Med. Surg. 20 (2017) 87–91.
[17] F. Simonacci, N. Bertozzi, M.P. Grieco, E. Grignaffini, E. Raposio, Procedure, applications, and outcomes of autologous fat grafting, Ann. Med. Surg. 20 (2017) 49–60.
[18] E. Raposio, N. Bertozzi, Isolation of ready-to-use Adipose-derived Stem Cell (ASC) pellet for clinical applications and a comparative overview of alternate methods for ASC isolation, Curr. Protoc. Stem Cell Biol. 16 (2017) 1F.17.1–1F.17.
[19] N. Bertozzi, M. Pesce, P.L. Santi, E. Raposio, Oncoplastic breast surgery: comprehensive review, Eur. Rev. Med. Pharmacol. Sci. 21 (2017) 2572–2585.
[20] X. Zhang, Biomedical engineering for health research and development, Eur. Rev. Med. Pharmacol. Sci. 19 (2015) 220–224.
[21] X. Yao, L. Li, X.L. He, L. Cui, W. Kuang, M. Tang, Activation of β-catenin stimulated by mechanical strain and estrogen requires estrogen receptor in mesenchymal stem cells (MSCs), Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 3149–3155.
[22] K. Zhang, F. Zhang, W. Zhao, et al., Effects of parathyroid hormone-related protein on osteogenic and adipogenic differentiation of human mesenchymal stem cells, Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 1610–1617.
[23] T. Simsek, G. Duruksu, A. Okçu, et al., Effect of hypothyroidism in the thyroidectomized rats on immunophenotypic characteristics and differentiation capacity of adipose tissue derived stem cells, Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 617–629.
[24] S. Agacayak, B. Gulsun, M.C. Ucan, E. Karaoz, Y. Nergiz, Effects of mesenchymal stem cells in critical size bone defect, Eur. Rev. Med. Pharmacol. Sci. 16 (2012) 679–686.
[25] M.A. Puglisi, N. Saulnier, A.C. Piscaglia, et al., Adipose tissue-derived mesenchymal stem cells and hepatic differentiation: old concepts and future perspectives, Eur. Rev. Med. Pharmacol. Sci. 15 (2011) 355–364.
[26] A.C. Piscaglia, C. Di Campli, P. Pola, A. Gasbarrini, When biology bursts into the clinic: stem cells and their potential, Eur. Rev. Med. Pharmacol. Sci. 5 (2001) 151–154.
[27] R. Bracaglia, M. D'Ettorre, S. Gentileschi, G. Mingrone, D. Tambasco, Multiple lipomatosis after stem cell transplant and chemotherapy: a case report, Eur. Rev. Med. Pharmacol. Sci. 18 (2014) 413–415.
[28] E. Cigna, D. Ribuffo, V. Sorvillo, et al., Secondary lipofilling after breast reconstruction with implants, Eur. Rev. Med. Pharmacol. Sci. 16 (2012) 1729–1734.
[29] Directive 2001/83/ec of the European Parliament and of the Council, (6 November 2001) http://ec.europa.eu/health//sites/health/files/files/eudralex/vol1/dir_2001_83_consol_2012/dir_2001_83_cons_2012_en.pdf.
[30] Directive 2009/120/EC: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:242:0003:0012:EN:PDF.
[31] Overview of Comments Received on 'Reflection Paper on Classification of Advanced Therapy Medicinal Products' (EMA/CAT/600280/2010 Rev. 1). EMA/224106/2015.
[32] European Medicines Agency, Patient Health Protection, EMA/129056/2013 (4 April 2013).