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Use of nanoparticles in cell tracing in advanced therapies: possibilities and challenges for clinical application

Uso de nanopartículas no rastreamento de células em terapias avançadas: possibilidades e desafios para a aplicação clínica

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ABSTRACT

Introduction: Regulation and introduction of new advanced cellular technologies in clinics requires better knowledge on the used cells. One of the major challenges is the difficulty to accurately determine the mechanisms of cell activities when submitted to advanced therapies. **Objective:** Understand, using the cell tracking methods, the tissue distribution and the molecular response of cells exposed to advanced therapies. **Methods:** This work was done as an integrated review of data collected in international data-bases and of legislations concerning the clinical use of nanoparticles. **Results:** Nanotechnology is a very useful tool for cell tracking in vivo, and several nanoparticles have been already approved for clinical use; they can be incorporated into cells and visualized by magnetic resonance imaging. **Conclusions:** The perspectives on the use of nanomedicines for cellular tracking in medical clinic are promising, requiring elaboration and approval of laws, guides and norms to orient the industrial sectors, regarding the nanomaterials characterization and potential risks to human and animal health, as well as to the environment.

KEYWORDS: Magnetic Nanoparticles; Cell Tacking; Stem Cells; Advanced Therapies; Nanomedicine

RESUMO

Introdução: Para uma regulamentação específica e implementação das novas tecnologias avançadas na clínica médica, é preciso refinar o conhecimento sobre as células utilizadas. Um dos maiores desafios está na dificuldade em determinar os mecanismos de funcionamento das células após a terapia. **Objetivo:** Compreender, usando os métodos de rastreamento celular em pacientes, a distribuição e a função de células usadas em terapias avançadas. **Método:** Este trabalho foi elaborado pelo levantamento de artigos científicos nas bases de dados internacionais e da legislação vigente sobre a utilização clínica das nanopartículas. **Resultados:** Constatamos que a nanotecnologia já é uma ferramenta de grande utilidade, pois diversas nanopartículas magnéticas já são aprovadas para uso clínico; elas podem ser incorporadas por células e visualizadas por imagens de ressonância magnética. O presente texto discute as perspectivas sobre uso de nano-medicamentos para rastreamento celular na clínica médica. **Conclusões:** A utilização das nanopartículas necessita a elaboração e aprovação de leis, guias e normas que orientem a indústria, como outros setores, sobre a caracterização dos nanomateriais e potenciais riscos à saúde humana, animal e ao meio ambiente.

PALAVRAS-CHAVE: Nanopartículas Magnéticas; Rastreamento Celular; Células-tronco; Terapias Avançadas; Nanomedicamentos

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INTRODUCTION

For the implementation of new proposals and new advanced cell therapy products, clinical trials demonstrating the absence of adverse effects and the presence of desired therapeutic effects initially performed in phases I and II are required. Information on the distribution of transplanted cells, both temporal and spatial, are fundamental to understand the effects of these new therapies. In view of that, nanoparticles have been extensively explored for this purpose.

In recent years, nano science and technology have drawn a great deal of attention in various areas of knowledge, due to the expectation of the impact that nanostructured materials can have on society. Because of their unique physicochemical properties, a plethora of everyday products incorporated nanomaterials into their production, like stain-and wrinkle-resistant fabrics; microprocessors; germ-resistant toys; long-life batteries; LED lights and biodegradable plastics. In addition, a number of products with application in the human body have also been developed, such as cosmetics and sunscreens, and even some of internal application such as medicines, heart valves, pacemakers, orthopedic catheters and implants[1,2].

There are no nanoparticles approved for human use specifically produced for cell tracking, but there are a number of magnetic nanoparticles approved for diagnosis or therapy, which can alternatively be used for cell tracking. In the present text, we will analyze the perspectives of the use of nanoparticles in the tracking of cells, especially on the methodologies that can be readily used in the medical practice. Furthermore, we will also discuss the need to develop and approve legislation to guide the industry, like in other sectors, on the characterization and potential risks of nanomaterials.

METHOD

For this review, we consulted the scientific literature related mainly to clinically approved magnetic nanoparticles and the tracking of cells in therapies, along with the applicable legal framework. The search for scientific papers was carried out in the PubMed and Google Scholar databases. The search for the legal framework was carried out in the publications available on the electronic portal of the Brazilian National Sanitary Surveillance Agency (Anvisa), the Chamber of Deputies and the Federal Government. We used keywords like nanoparticles, nanotechnology, nanoparticles, superparamagnetic iron oxide nanoparticles, cell labeling, cell tracking, in vivo tracking, molecular imaging technology, nanoparticle toxicity, advanced therapies, stem cells, both in the Portuguese and English languages).

RESULTS AND DISCUSSION

Nanotechnology

A first aspect that draws attention to nanotechnology is the very definition of the term: there is no consensus about it because it is a diversified area that covers a large set of technologies. Currently, nanomaterials are defined primarily by their size. Some authors consider as nanomaterials only structures of 1 to 100 nm in size in one of the dimensions[3]; others accept larger sizes, as in the definition of the International Standards Organization (ISO), which states that nanotechnology includes the control of matters and processes at the nanoscale, typically but not exclusively below 100 nm in one or more dimensions[4]. In Brazil, according to Bill (PL) n. 6.741, of 2013, the term nanotechnology refers to the "handling of materials on a scale ranging from 1 to 100 nm in at least one of its dimensions". It also defines the term nanomaterial, which refers to "material with one or more external dimensions, or internal structure, based on the nanoscale, which may exhibit new characteristics". These Brazilian definitions reflect the difficulty in defining the term, since nanotechnology is defined by the maximum size of 100 nm, and the nanomaterial is accepted within the nanometric dimension, thus allowing materials with up to 1,000 nm. A precise and international definition is important for regulation and marketing (import and export) of materials produced from nanoscale materials.

In an article called "do not define nanomaterials", the author argues that a definition based on the science of nanomaterials is needed, focusing on the new properties and phenomena observed, not just in the definition of size[5]. In the present text we will use the term nanomaterials for those materials produced at the nano scale, that is, those between 1 and 1,000 nm.

Nanoparticles

Nanomaterials can be divided into particles or fibers that have the nanometric range in one of their dimensions. Nanoparticles that are defined as solid colloidal particles (nanospheres) or vesicular types (nanocapsules). New synthetic techniques have allowed the production of nanoparticles with other non-spherical shapes, such as prism, hexagon, cube, among others[6].

Use of nanoparticles in the medical field

Nanomedicine is a term of contemporary medicine that has emerged with the combination of medicine and nanotechnology. It consists of the use of nanomaterials in therapeutic and diagnostic methods. In the field of nanomedicines, a size ranging from a few nanometers to 1,000 nm in diameter has been well accepted. In practice, the useful range of nanomedicines usually falls within the range of 5-250 nm[7]. Some drugs have seemed to be less toxic and more efficient when nanoencapsulated than in their free state[8,9]. Additionally, the use of stem cells as nanoencapsulated drug carriers has been investigated, especially in the treatment of glioma, since stem cells, like mesenchymal cells, are typically able to cross the blood-brain barrier and have tropism for tumor cells[10,11,12,13].

Another possibility of the use of nanoparticles in medicine is their internalization by stem cells for in vivo tracking, which is the main focus of this text. The mechanisms of the new cell

therapies may be understood in part by tracking the cells after transplantation in the patient. Several studies, including those in our group, have efficiently shown the labeling of cells[10,15,16].

Available methods of cell labeling

There are different ways to proceed to cell labeling. Essentially, they are divided into two categories: direct and indirect labeling. Indirect labeling includes the genetic modification of the cell so that it expresses a signal molecule or to increase affinity for contrast agents[17,18]. The potential risks derived from genetic manipulation hinder the clinical approval of this procedure. Direct labeling consists of incubating the cells directly with markers through a simple incorporation procedure, without the need for genetic manipulation. There are different direct labeling techniques that allow the incorporation of the markers both during cell culture and in fresh cells[19,20,21]. This latter is essential when cell therapy is planned without cell pre-cultivation.

The most commonly used markers are radionucleotides, fluorescent and/or magnetic nanoparticles. Radionucleotides are radiation-emitting substances that can be visualized by positron emission tomography (PET) and single photon emission tomography (SPECT). Several radiopharmaceuticals are approved for in vivo treatment and diagnosis and may be used for cell tracking. The major shortcoming of the technique is the half-life of the markers, which is generally reduced to a few hours. This has two consequences: the first is that the in vivo cell tracking time is short and the second is the short time available to transport the radioisotopes, because they are produced by specific equipment such as particle accelerators or nuclear reactors. In Brazil, there are few radiopharmaceutical producing units. The main one is located in São Paulo, at the Energy and Nuclear Research Institute, which produces 38 different radiopharmaceuticals[22].

The labeling of cells with fluorescent nanoparticles is interesting in preclinical research, as it is a low-cost technique with high availability of fluorescent markers[6]. However, this technique cannot be applied clinically because the fluorescent light has a low penetration capacity on the surface of the body, limited to millimeters.

Compared to the techniques described above, magnetic resonance imaging (MRI) is today the most widely available and clinically applicable technique, through the labeling of the cells with magnetic nanoparticles. This technique presents high spatial resolution and the advantage that functional patient data can be collected simultaneously with the tracking data. One of the major shortcomings of this technique is its low sensitivity when compared to fluorescent or radioactive markers. Several paramagnetic nanoparticles of metal ion binding complexes, such as gadolinium[23] and iron oxide superparamagnetic nanoparticles (SPIONs)[24] are approved for diagnosis or therapy with patients. Labeling and tracking cells using gadolinium nanoparticles or SPIONs have been shown to be effective[25,26]. However, SPIONs are the most studied nanoparticles for this purpose, among other factors because their renal clearance is slower and because they present a stronger signal by MRI compared to gadolinium-based contrast agents[27,28]. In a recent study, a double-contrast method was proposed using SPION and gadolinium, which would detect both cell localization and death[29].

Overall, the main shortcoming of direct labeling techniques is the possibility of cell marker leakage, leading to signal loss and/or diffusion, and yielding unreliable results[6,18,30]. Therefore, despite several authors describing the possibility of long-term tracking - up to months[26] - we recommend that patient tracking be performed for shorter periods, from days to a few weeks, to prevent misinterpretation.

The ideal nanoparticle for clinical application does not exist, but it should be biocompatible, non-toxic, stable at physiological pH, remain retained only in the labeled cells and be rapidly eliminated after cell death. Specifically for the tracking using MRI, an important feature is the generation of nanoparticles with high magnetization due to the low sensitivity of the technique. To date, there are no markers with all these characteristics, and therefore, the studies explore the use of nanoparticles already available and approved for human use.

Clinically approved magnetic nanoparticles

Feridex/Endorem SPIONs (120-180 nm in diameter) are produced for liver tumor detection, available from AMAG Pharmaceuticals/Guerbet. Feridex and Endorem are the same product with different trade names: while Feridex was produced in several countries, such as USA, Japan, Argentina, China, South Korea, Endorem was mainly distributed in Europe and in Brazil. However, these products have been discontinued and are no longer produced. Combidex/Sinerem (15-30 nm) nanoparticles are approved for lymph node tumor diagnosis, available from AMAG Pharmaceuticals/Guerbet, but they have also been discontinued. Resorvist (45-60 nm) enables the detection of liver tumor and Supravist (21 nm) is used for angiography, both marketed by Bayer Schering. Nevertheless, Resorvist has been discontinued. Exceptionally, Feraheme (30 nm) is not used for diagnosis, but as a supplement in the treatment of anemia. It is produced by AMAG Pharmaceutical. All nanoparticles mentioned above are based on iron oxide. Recently a new formulation of the gadolinium-based Clariscan (11-20 nm) for microvasculature tumor imaging has been approved for human use by GE Healthcare. Furthermore, gadolinium-based products are also available under trade names like Dotarem and Omniscan, which are indicated to visualize areas of blood-brain barrier rupture or abnormal vascularization in the brain, spinal cord and associated tissues (Guerbet and GE Healthcare, respectively). Magnevist (Bayer Schering) was the first intravenous contrast agent to become available for clinical use and indicated to display abnormal vascularization in the brain and body. It is noteworthy that the tests performed with nanomedicines are the same as those conducted with conventional drugs, and there are no specific tests for drugs based on nanoparticles.

The discontinuation of nanoparticle production has affected the studies on cell tracking, since a large number of studies, including our group, were published investigating cell labeling

with materials that are no longer commercially available. It is important to note that the discontinuation occurred mainly due to economic factors. As far as we know, there have been no cases of toxicity that led to the discontinuation of the products.

This shows a niche for the development of new nanoparticles specific for cell labeling and tracking. Currently, several companies market nanoparticles that are not clinically approved for preclinical studies, however it is not clear if there is the intention of future clinical approval.

Through the verification of the medicinal products registered in Anvisa, we observed that only Endorem, Dotarem and Omniscan, of all the nanoparticles mentioned above, are registered as medicines in Brazil. Endorem is registered under number 106140022 and its registration expired in September 2005. Dotarem is registered under number 149800016 and its expiration date is August 2021, while Onmiscan is registered under number 183960003 and it expired in December 2017[31]. This small number of magnetic nanoparticles approved in Brazil limits the options of Brazilian researchers, both for possible tracking in medical practice and in preclinical studies, because they cannot be readily imported.

Use of approved nanoparticles in cell tracking

To clarify how the nanoparticles approved as medicines can be used in cell tracking, we will take Feridex as an example. It is used to detect liver tumors. For diagnosis, Feridex prepared in a 5% dextrose solution is administered as a drip infusion for about 30 min. One hour after the intravenous injection, the iron oxide particles are absorbed by the reticuloendothelial cells of the liver and spleen, with an approximate uptake of 80% and up to 10%, respectively. The tumor tissue does not incorporate the particles and thus remains, by MRI, with native contrast intensity. A small amount of nanoparticles can be detected in other organs like kidneys, liver and brain[32]. According to the package insert, Feridex's nanoparticle iron enters the normal cycle of body iron metabolism, evidenced by transient increases in serum iron values one day after administration and increase in serum ferritin values 7 days after dosing. The amount of iron contained in a single dose is approximately 39 mg for a 70 kg individual, which is less than 1/5 of the amount of iron contained in a blood bag for transfusion.

Also in Feridex's package insert, in clinical trials, anaphylactic and allergic adverse events occurred in 11 of 2,240 (0.5%) patients. These events include dyspnea, other respiratory symptoms, angioedema, generalized hives and low blood pressure. Acute pain in the back, legs or groin occurred in some patients. Fifty-five of 2,240 (2.5%) patients presented pain intense enough to cause interruption of the infusion. In most patients, the symptoms appeared within 1 to 15 minutes.

The single recommended dose of Feridex is 0.56 mg iron per kilo of body weight. This means that a 70 kg patient will receive approximately 39 mg of iron intravenously. For cell labeling, like for mesenchymal stem cells, an amount of 10 pg of iron incorporated per cell is considered sufficient for in vivo tracking[33]. The amount of cells injected into cell therapies varies greatly according to the cell type, the disease and the route of injection. Assuming a therapy in which 1 x 10^6 mesenchymal stem cells per kg body weight are used, in a 70 kg patient, 0.7 mg of iron incorporated into the cells will be administered. In other words, the amount of iron injected together with the cells is almost 56 times lower than the recommended dose for the diagnosis using a single dose. This same rationale can be used for several other magnetic nanoparticles approved for human use and experimentally incorporated by in vitro cells.

In 2009, a pilot clinical study was conducted to label cells with Endorem for MRI screening in healthy patients. The results showed that peripheral blood mononuclear cells can be labeled without affecting their viability, migratory capacity and cytokine production (interleukin [IL] -1, IL-6, IL-10, IL-12p70, and tumor necrosis factor [TNF]) for up to 72 hours after labeling. Healthy patients received labeled cells intramuscularly or intravenously. It was possible to visualize the cells for up to 7 days, and they were mainly found in the liver and spleen. In the conclusion of the study, the authors state that the technique was efficient and safe[34].

Although the toxicity of nanomedicines is tested in humans for approval, it is important to evaluate possible toxic effects on cells. The presence of nanoparticles cannot interfere with the potential of cell therapy, therefore, studies continue to characterize the effects of nanoparticles on cell biology, including viability, influence on migration, proliferation, differentiation and grafting. Many preclinical studies have shown the use of the nanoparticles safely, without affecting a number of cell parameters[15,35,36]. Others have shown relatively low toxicity, indicating induction of cell stress, changes in gene expression, decrease in the proliferation rate or promotion of a pro-inflammatory environment[15,37,38]. Furthermore, the toxic effect is not always due to the presence of the nanoparticles themselves, but rather because of how they are pre-engineered to induce incorporation by the cells.

To evaluate the effect of nanoparticles on labeled cells, even if they have already been approved for human use, some in vitro cytotoxicity tests need to be carried out. In mesenchymal stem cells, for example, we suggest that possible changes in the potential "stem" of these cells be assessed. According to the International Society of Cellular Therapy, mesenchymal cells should differentiate in vitro into adipocytes, osteocytes and chondrocytes, express CD105, CD73 and CD90, and not express CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR. In addition, we suggest that at least tests be done to characterize the rate of proliferation and cell death, which can be applied to any cell type. Some more advanced tests such as gene expression, cell stress, among others, can also be used to determine cytotoxicity in different cell types.

It is important to note that, even with the possibility of some level of toxicity, this problem can be overcome, since only a sample of cells can be used for tracking. In this sense, there is a

need for studies aimed at determining the minimum number of cells to be labeled, which should take into account mainly the cell type and the route of administration.

The data discussed in this section clearly reflect the possibility of using nanoparticles approved for human use in the tracking of cells in patients. However, it is essential that there be regulation of nanomedicines, regardless of the type of application.

Regulatory frameworks in nanotechnology

Despite the growing number of nanotechnology-containing products coming to the market, assessing the risks of nanoparticles to health and the environment is still a challenge. In the USA, the Food and Drug Administration (FDA) and in Europe the European Animal Medicines Agency (EMA) regulate the sanitary safety of nanotechnology products for human and animal use. The FDA has issued guidelines for the industry. They do not establish regulatory definitions, but are intended to guide and advise the industry and others on safety, efficacy or public health impact as well as on post-marketing control[39]. Similarly, the EMA has published several documents aimed at standardizing and guiding the industry and other stakeholders. The European Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), which produces reports on emerging or newly identified health and environmental risks, has identified that the biological evaluation of nanoparticles and/or products incorporating nanoparticles should be done at every case[40].

In Brazil, the first bills aimed at the regulation of nanotechnologies date from 2005 and 2010 PL n. 5.076/2005 and PL n. 131/2010, respectively. Both were archived. Beginning in 2012, other efforts were made by the government to boost the development of the topic. The National Strategy for Science, Technology and Innovation (ENCTI) 2012-2015 included nanotechnology as one of the "priority programs for the future-bearing sectors". The topic of nanotechnology is still considered as a strategic area in the ENCTI 2016-2019, which was released in 2016, but still under discussion and not approved in its final version.

The Ministry of Science, Technology, Innovation and Communications (MCTIC), through ordinance n. 245, of April 5, 2012, established the National System of Laboratories in Nanotechnology (SisNano), whose main objectives are to encourage research, development and innovation in this sector. Also in 2012, the MCTIC, along with seven other Ministries, among them the Ministry of Health, through ordinance n. 510, of July 9, established the Interministerial Committee on Nanotechnologies, with the purpose of advising several Ministries, as well as improving policies, guidelines and actions aimed at the development of nanotechnologies in Brazil. In 2014, the Interministerial Committee on Nanotechnology approved Brazil's accession to the NanoReg European project, which involves European countries and others like Australia, Canada, South Korea, the United States and Japan. NanoReg is an international project that aims to provide technical and scientific support to all regulatory issues in nanotechnology.

Since 2013, two bills are under discussion in the Brazilian Chamber of Deputies. They are currently being processed. PL n. 6.741/2013 "provides for the National Policy on Nanotechnology, research, production, destination of waste and the use of nanotechnology in the country, and provides other measures"[41]. This project is linked to PL n. 5.133/2013, which "regulates the labels of nanotechnology products and nanotechnology-related products"[42]. This project addresses the right of access to information provided by the Consumer Defense Code.

A legal framework on products containing nanotechnology is important, even because of the need to delegate power to agencies and regulatory bodies like Anvisa.

Regulation of nanotechnology by Anvisa

Anvisa plays a fundamental role as the body responsible for supervising the production and marketing of health-related products, such as food, medicines and cosmetics. After 2013, the nanotechnology theme became part of the regulatory agenda of the agency. In the 2013-2014 agenda, topic 112 addressed "nanotechnology related to products and processes subject to sanitary surveillance". Its objective was "to promote sanitary regulation related to technological innovations arising from nanotechnology, considering its importance as an area of future-bearing innovation, as well as its potential risks". In compliance with the topic, ordinance n. 1.358, of August 20, 2014, was published. It established the Internal Nanotechnology Committee of Anvisa. Among the attributions of the Committee is the elaboration of norms and guides for the evaluation and control of products that use nanotechnology. For the biennium 2014-2016, the regulatory agenda has kept the topic nanotechnology as one of its priorities. The agenda for the coming years is currently under discussion[43]. However, despite the efforts, as far as we know there are no standards or guidelines guiding the industry and others on the development and risks of nanotechnology-containing products in Brazil, although this has already been done by the FDA in the USA and the EMA in the European community.

Because of this lack of specific regulation for nanotechnology, specific tests to guarantee the public safety of medicines containing nanomaterials are not required at Anvisa's time of registration. Another consequence is the lack of clarity for consumers, since there is no obligation to inform them about the presence of nanoparticles in the medicines, neither on the package insert or on the label. It is worth remembering that the bill that regulates the labeling is being processed since 2013. Therefore, there is the possibility of having several registered products not identified as nanotechnology products.

Specific tests in the field of nanotechnology require specialized people, sophisticated equipment and quality assessment that cover parameters other than particle size. These parameters still need to be defined. Furthermore, a risk assessment on a case-by-case basis, as recommended by the US and European regulatory agencies, is important.

CONCLUSIONS

Cell tracking enables better understanding of the functioning mechanisms of advanced cellular therapies, thus facilitating the approval of several therapies still in the experimental phase. For this, it is possible to use magnetic nanoparticles marketed as medicines to label and track transplanted cells. We suggest that there should be no specific regulation for the use of these nanomedicines in cell tracking, since these products are approved in accordance with current standards, and the amount used for tracking is much lower than that already approved for other clinical uses. However, it is important to establish regulations for nanotechnology drugs in general, ensuring their use in a safe and environmentally responsible fashion. Specific tests must be carried out by specialized personnel and equipment capable of characterizing the various nanomaterials and verifying their toxicity. Parameters of nanomaterials, in addition to particle size, still need to be defined. Additionally, it is important to establish ways to dispose of and destroy materials to minimize potential risks. In this sense, we can point out some early efforts carried out within MCTIC and Anvisa, but laws, guidelines and standards orienting the industry, like other sectors, need to be drafted and approved by the responsible bodies, and the consumers' direct access to information must be ensured.

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Conflict of Interest

Authors have no potential conflict of interest to declare, related to this study's political or financial peers and institutions.