REVIEW

Theranostics and Molecular Imaging: new concepts and technologies for drug development

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ABSTRACT

Some of the bases, challenges and expected outputs of the Molecular Magnetic Resonance Imaging and the Theranostics in the near future are presented in this paper. The Molecular Imaging, which evolution in the last years is truly surprising, is becoming a real tool for decreasing the cost and accelerating the stages of drug discovery and development processes. The new Theranostic platforms and the integration of imaging technologies have opened novel and revolutionary opportunities in medicine and biotechnology-based pharmaceutical industries. The strategy of joining therapeutic and imaging agents into one nano platform has the potential to diagnose disease and to treat and monitor the therapeutic response *in vivo* at molecular level. It simultaneously enhances the control, evaluation and optimization of drug delivery, release and its efficacy. These theranostic formulations are also increasing the Molecular Magnetic Resonance sensibility to some picomoles. Remarkably, it is widely considered that the conjugation of paramagnetic complexes with versatile macromolecules and natural nanostructures is the most important and fastest way to obtain original theranostic formulations. The optimum use and wider applicability of Molecular Imaging relies on the best possible design of the pre-clinical and clinical trials taking into account the imaging technologies from the very same beginning. The complex problem of personalized medicine, combined therapy, and the synchronization of the therapy of choice with diagnosis may find an outstanding solution in the Theranostics approach.

Keywords: theranostics, drug development, molecular imaging

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RESUMEN

La teranóstica y las imágenes moleculares: Nuevos conceptos y tecnologías para el desarrollo de fármacos. En este artículo se discuten algunas de las bases, los retos y el uso de las imágenes moleculares y la teranóstica en un futuro cercano. Las imágenes moleculares, cuya evolución en los últimos años ha sido sorprendente, se han convertido en verdaderas herramientas para disminuir los costos y acelerar todas las etapas de los procesos de descubrimiento y desarrollo de nuevos fármacos. La integración de las actuales plataformas de la teranóstica con las tecnologías de imágenes ha abierto oportunidades novedosas y revolucionarias en la medicina y las industrias biotecnológicas y farmacéuticas. La estrategia de unir agentes diagnósticos y terapéuticos en una nanoplataforma ofrece la posibilidad de diagnosticar, tratar y monitorear la respuesta terapéutica *in vivo* a nivel molecular. Esto permite mejorar simultáneamente la evaluación, la optimización, el control y la eficacia de la distribución y liberación de los medicamentos. Estas formulaciones teranósticas también han incrementado la sensibilidad de las imágenes moleculares de resonancia magnética hasta algunos picomoles. Como conclusión se afirma que la conjugación de complejos paramagnéticos con macromoléculas y nanoestructuras naturales flexibles es la vía más importante y rápida para obtener nuevas formulaciones teranósticas. Para optimizar los ensayos preclínicos y clínicos es necesario que desde el inicio se diseñen teniendo en cuenta las posibilidades de las tecnologías de imágenes. Los problemas complejos asociados con la medicina personalizada, la terapia combinada y la sincronización entre las acciones diagnósticas y terapéuticas encuentran una excelente solución en el campo de la teranóstica.

Palabras clave: teranóstica, desarrollo de drogas, imágenes moleculares

*I*ntroduction

Molecular Imaging, which evolution in the last years is truly surprising, is strongly connected to biology, chemistry and the pharmaceutical industry [1-18]. There are, at least, three main reasons to focus on Molecular Imaging regarding drug development: 1) the large sequence of steps from drug discovery through development, at a very high cost and being time-consuming; 2) the current scientific problem of systems biology; and 3) the recent acknowledgement of imaging as a branch of the Theranostics, *i.e.*, the relationship of therapy and diagnostic at nanoscale level.

Of them, the first reason relies on the values of Molecular Imaging methods as powerful technologies lowering costs and accelerating the steps from drug discovery through pharmaceutical development. They provide simultaneously all the information obtained by the previous complex sequence of steps from the laboratory research to production and the final clinical use [12-18]. The requirements of the pharmaceutical industry are the final purpose catalyzing the advances of Molecular Imaging science and technologies. The second comprises one of the main questions of

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1. Cabal C, González E, Torne Y, Rojas A. Micro-magnetic and molecular magnetic resonance imaging in modern biotechnology and pharmacy. Biotecnol Apl. 2009; 26:304-14.

2. Molecular and Cellular MR Imaging. Baltimore, MD: CRC Press. Johns Hopkins University School of Medicine; 2007.

3. Terreno E, Castelli DD, Viale A, Aime S. Challenges for molecular magnetic resonance imaging. Chem Rev. 2010;110: 3019-42.

systems biology: how to transform the molecular information from cells, tissues, organs and organism into understandable knowledge on complex phenomena? [19, 20]. In fact, the equivalencies between molecular and classical imaging methods allow us to obtain, at the same time, rich *in vivo* information about structure and functionality at all the possible life organization levels, from a single cell through an entire organism (Figure).

The basic idea of Theranostics consists on creating a nanocapsule containing a drug and an imaging contrast agent as a single formulation, supporting the follow-up *in vivo* of the action of the drug [21-23] (Figure). Theranostic ideas and methods are producing a revolution, creating original technology platforms and challenging the established concepts. Three fields of drug development are covered by imaging as Theranostics: delivery, release and efficacy evaluation. Indeed, imaging was remarked in the FDA's Critical Path Initiative as a key technology to overcome the bottleneck in the development and registration of innovative medicines [24].

The Positron Electron Tomography (PET), Single Photon Emission Computed Tomography (SPECT), Computer Tomography (CT), ultrasound (US), and the Magnetic Resonance Imaging (MRI) together with a diversity of optical methods are the most important imaging platforms for drug development (Figure). The advantages and restrictions of the different imaging modalities have been addressed in the scientific literature [2-6, 11-13, 18]. These technologies have the potential to enhance our understanding of disease and drug activity during the pre-clinical and clinical drug development phases. They are, in general, complementary rather than competitive techniques [3, 5, 11, 13, 15]. That's why it is indispensable to affirm that the best approach to consider a specific problem is the multi-modal imaging; this imaging hybrid has the potential to provide the desired results.

The needed amount of suitable animal models is one of the restrictions and the source of increased costs during drug development. Imaging methods can help on replacing, reducing and refining the use of animal models imaging in drug delivery and release studies [13].

Imaging technologies must begin at early stages of the research process in order to support all the critical phases of drug discovery and development such as the 'proof of biology' and the 'proof of concept' [24, 25]. In fact, there have been recently summarized [26] the imaging methods possibilities and tasks in drug discovery and development framework as: 1) identifying the disease phenotype, 2) pre-selection of target populations, 3) monitoring in real time dose and dose scheduling (pharmacodynamics and pharmacokinetics), 4) validation of drug efficacy and time course of efficacy, 5) individual patient management, and

4. Villaraza AJ, Bumb A, Brechbiel MW. Macromolecules, dendrimers, and nanomaterials in magnetic resonance imaging: the interplay between size, function, and pharmacokinetics. Chem Rev. 2010;110:2921-59.

5. Massoud TF, Gambhir SS. Molecular imaging in living subjects: seeing fundamental biological processes in a new light. Genes Dev. 2003;17:545-80.

6. Gross S, Piwnica-Worms D. Molecular imaging strategies for drug discovery and development. Curr Opin Chem Biol. 2006; 10:334-42.

7. Glunde K, Artemov D, Penet MF, Jacobs MA, Bhujwalla ZM. Magnetic resonance spectroscopy in metabolic and molecular imaging and diagnosis of cancer. Chem Rev. 2010;110:3043-59.

8. Hoehn M, Himmelreich U, Kruttwig K, Wiedermann D. Molecular and cellular MR imaging: potentials and challenges for neurological applications. J Magn Reson Imaging. 2008;27:941-54.

9. El-Deiry WS, Sigman CC, Kelloff GJ. Imaging and oncologic drug development. J Clin Oncol. 2006;24:3261-73.

10. Weissleder R, Mahmood U. Molecular imaging. Radiology. 2001;219:316-33.

11. Cherry SR. *In vivo* molecular and genomic imaging: new challenges for imaging physics. Phys Med Biol. 2004;49:R13-R48.

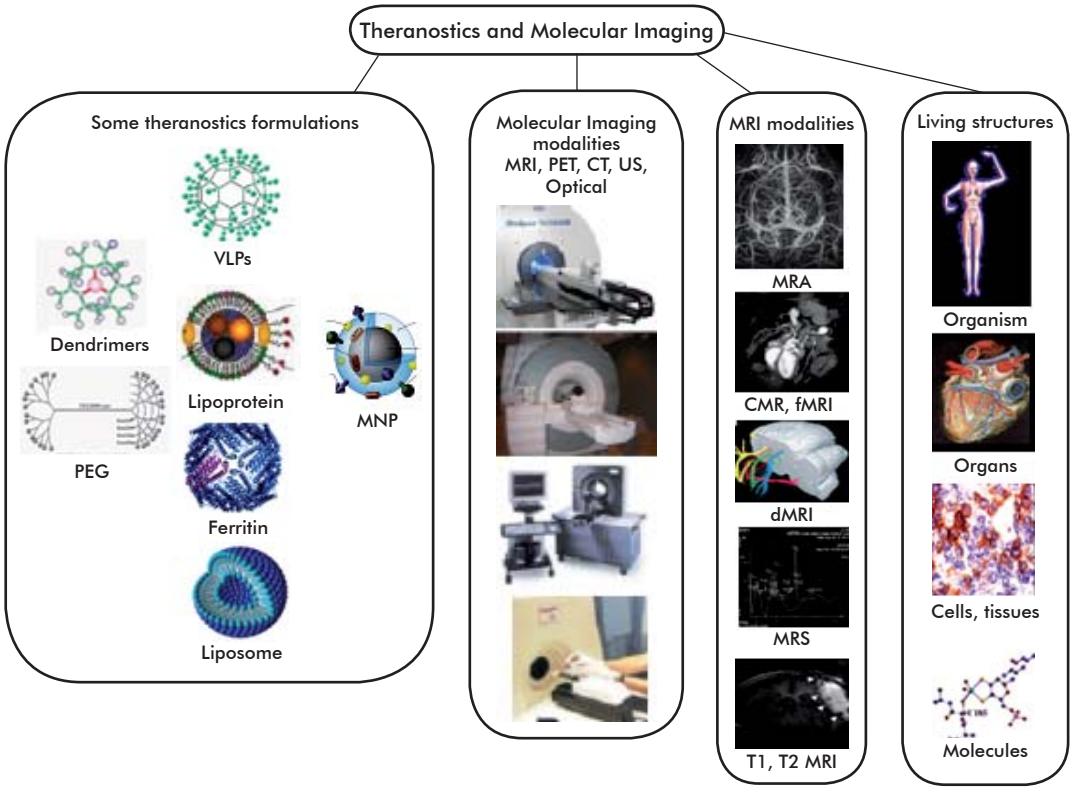

Figure. The Theranostics and Molecular Imaging techniques interaction. Theranostics (*i.e.*, the relationship of therapy and diagnostic at nanoscale level) integrate formulation platforms as dendrimers, polyethylene glycols (PEG), virus-like particles (VLPs), liposome, lipoprotein, ferritin, magnetic nanoparticles (MNP) and similars, with imaging technologies as magnetic resonance imaging (MRI), positron emission tomography (PET), computer tomography (CT), ultrasound (US) and others, to open novel and revolutionary opportunities in the biotechnological and pharmaceutical industries. The correspondence between the molecular and the classical imaging methods supports to obtain rich *in vivo* information about the structure and functionality of all the living levels. CMR: cardiovascular magnetic resonance imaging. dMRI: diffusion MRI. fMRI: functional MRI. MRA: magnetic resonance angiography. MRS: magnetic resonance spectroscopy. T1, T2 MRI: T1- and T2-weighted MRI.

6) imaging to guide biopsy sites for genomic and proteomic assays.

Additionally, choosing the imaging modality during drug development depends primarily on the specific question to be addressed. However, of them, only MRI is able to bring anatomical, physiological and molecular information of a living system in the same study. One of the most important and less appraised advantage of MRI is the possibility to study a large assortment of different animal models, and also, to be employed in the clinical studies. This advantage permits the scale of MRI procedures from preclinical to clinical research without any essential change in the experimental conditions with the subsequence benefit of lower costs and time reduction. In this review, considerations will focus mainly on molecular MRI (mMRI) and its connections with drug discovery and development. Some of the principal challenges, limitations and possible outputs of mMRI related to the Theranostics are also discussed.

Theranostics and Molecular Imaging

The MRI has an anatomical resolution record of about 10-50 micrometers, which is enough to study the structures typically addressed in biomedical research [2-6, 11-14, 24], while its sensibility is about 0.1 micromoles, yet far from the optimum [3, 5, 11, 12, 14, 18]. The contrast agent (CA) is one of the fields waiting for optimization to increase MRI sensitivity. In the annals of imaging, CA development has been steadily evolving to become a molecular magnetic marker or a magnetic label. At this moment, CA plays two evident and strong related roles in mMRI: to increase images' contrast and to be a molecular magnetic label to follow those different processes at all living levels in the neighboring areas of the labeled molecule. Furthermore, the molecular marker had increased the mMRI sensitivity and is expected that it can be in the range from nanomoles to some picomoles.

The mMRI sensitivity is related to the change of the relaxivity of the CA [2-4]. The increase of the relaxivity results in a greater image contrast or the ability to detect the CA at a lower concentration. The relaxivity depends on the CA complex structure (ligand field strength and symmetry) and its kinetics and molecular interaction. The molecular interactions modulate the symmetry and the strength of the ligand field of the CA complex. This idea is essential to understand the action mechanism of the CA as a molecular MRI marker. When the CA interacts with its surroundings, the relaxivity changes and the MRI contrast becomes different. One of the most important problems, from the chemical point of view, is to modify the complex structure to obtain the biggest relaxivity in order to achieve the highest sensitivity.

Theranostics formulations could include, besides the CA, several other agents like therapeutic, target, and improving permeation agents [2-4, 21-23, 27-30]. This means that it is possible to improve simultaneously the biodistribution properties and to follow the therapeutic achievement imaging by mMRI with Theranostic formulations [1-5, 27-30]. This is one of the most essential concept developments of the last decade. More and more the new therapeutic formulation will contain other agents enhancing the delivery, releasing and the efficacy of the drugs besides the CA as a marker, in order to visualize its localization and action inside the living body *in vivo*. The CAs are commonly classified into two main categories, *i.e.* endogenous or intrinsic and exogenous or extrinsic [1-4].

For the purpose of the mMRI, there are three principal ways to label the necessary CA or to obtain a magnetic marker. The first one consists in the management of the intrinsic magnetic pools, which are present inside the biological system. The second one is the use of extrinsic paramagnetic complexes and the super paramagnetic particle or magnetic nanoparticle (MNP). Finally, the existing natural nanoparticle can be labeled if conjugated to paramagnetic complexes. These natural nanoparticles could be either intrinsic or extrinsic agents.

The different magnetic states of hemoglobin (Hb) were the first intrinsic CA used for clinical and research purposes [1, 2]. The changes of the Hb magnetism have been the basis of the functional MRI (fMRI) for more than 20 years. The essential idea is that the oxyHb is a diamagnetic molecule and the deoxyHb is a strong paramagnetic complex. Then, when a physiological process is taking place at tissue level, the metabolism grows and, as a consequence, an increase of the rate of change of the oxy to deoxyHb also occurs. More oxyHb becomes to be deoxyHb and, as a result, the magnetism variation produces intensities changes in the MR images.

A more newer intrinsic genetic control mechanisms of contrast in order to follow by MRI physiological process at cellular level have been described in literature [31]. The basic idea of those genetic mechanisms consists on using a gene that manages the production of intracellular proteins, for example metalloprotein, which inside the cell forms a CA with the endogenous metal ions. Instead, the base of the semi genetic mechanism is to guide the synthesis of a cell surface protein which facilitates the introduction of extrinsic CA into the cell or into a convenient vesicle. It can be expected, that these and other different genetically controlled contrasts will be introduced into practice in the near future.

Another approach to provide contrast is a Magnetization Transfer (MT) technique termed Chemical Exchange Saturation Transfer (CEST) and the Paramagnetic Chemical Exchange Saturation Transfer (PARACEST) using the same principle of saturation transfer in the presence of an extrinsic paramagnetic complex. These endogenous mechanisms for MT are used clinically and exploit a pool of hidden water in some tissues [2-4, 32, 33]. These methods promise to become a good alternative to increase the contrast and to obtain molecular information for several applications (for more information see references [2-4, 32, 33].

The Gadolinium complexes were the first extrinsic CA with a very wide application in the clinical studies during the last 20 years [1-4, 9, 13, 27-29, 34-48]. There are two main categories of chelating ligand approved for clinical use, one a linear ligand, the diethylenetriaminepentaacetic acid (DTPA) and the other based on the macrocyclic ligand 1,4,7,10-tetra (carboxymethyl) 1,4,7,10-tetraazacyclododecane

12. Rudin M, Weissleder R. Molecular imaging in drug discovery and development. Nat Rev Drug Discov. 2003;2:123-31.

13. Pancholi K. A review of imaging methods for measuring drug release at nanometre scale: a case for drug delivery systems. Expert Opin Drug Deliv. 2012;9:203-18.

14. Rudin M. Noninvasive structural, functional, and molecular imaging in drug development. Curr Opin Chem Biol. 2009;13:360-71.

15. Achilefu S. Introduction to concepts and strategies for molecular imaging. Chem Rev. 2010;110:2575-8.

16. Hoffman JM, Gambhir SS, Kelloff GJ. Regulatory and reimbursement challenges for molecular imaging. Radiology. 2007;245:645-60.

17. Cai W, Rao J, Gambhir SS, Chen X. How molecular imaging is speeding up antiangiogenic drug development. Mol Cancer Ther. 2006;5:2624-33.

18. Glunde K, Pathak AP, Bhujwalla ZM. Molecular-functional imaging of cancer: to image and imagine. Trends Mol Med. 2007;13:287-97.

19. End of the interlude? [editorial]. Nat Biotechnol. 2004;22:1191.

20. Butcher EC, Berg EL, Kunkel EJ. Systems biology in drug discovery. Nat Biotechnol. 2004;22:1253-9.

21. Lammers T, Aime S, Hennink WE, Storm G, Kiessling F. Theranostic nanomedicine. Acc Chem Res. 2011;44:1029-38.

22. Ma X, Zhao Y, Liang XJ. Theranostic nanoparticles engineered for clinic and pharmaceutics. Acc Chem Res. 2011;44: 1114-22.

23. Caldorera-Moore ME, Liechty WB, Peppas NA. Responsive theranostic systems: integration of diagnostic imaging agents and responsive controlled release drug delivery carriers. Acc Chem Res. 2011; 44: 1061-70.

24. Wong DF, Tauscher J, Grunder G. The role of imaging in proof of concept for CNS drug discovery and development. Neuropsychopharmacology. 2009;34:187-203.

25. Kelloff GJ, Sigman CC. Cancer biomarkers: selecting the right drug for the right patient. Nat Rev Drug Discov. 2012; 11:201-14.

26. Blasberg R, Piwnica-Worms D. Imaging: strategies, controversies, and opportunities. Clin Cancer Res. 2012;18:631-7.

27. Kim JH, Park K, Nam HY, Lee S, Kim K, Kwon IC. Polymers for bioimaging. Polym Sci. 2007;32:1031-53.

28. Bellin MF. MR contrast agents, the old and the new. Eur J Radiol. 2006;60:314-23.

29. Yan GP, Robinson L, Hogg P. Magnetic resonance imaging contrast agents: Overview and perspectives. Radiography. 2007;13:e5-e19.

30. Sun C, Lee JS, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. Adv Drug Deliv Rev. 2008;60: 1252-65.

31. Westmeyer GG, Jasanoff A. Genetically controlled MRI contrast mechanisms and their prospects in systems neuroscience research. Magn Reson Imaging. 2007;25:1004-10.

(DOTA). The relaxivity obtained for this complex is about 3.9 $mM^{-1}s^{-1}$ [4]. New modifications were done to the Gd(DTPA) and Gd (DOTA) complexes' structure in order to change its biodistribution, its pharmacokinetics and to increase the relaxivity, *i.e.*, to raise the sensitivity [2-5, 27-29, 35-48]. For this purpose, the principal complex Gd(DTPA) is conjugated with many other kinds of polymer formation such as polyamine, polyethylene glycols (PEG), polysaccharides, PLL, and albumin. These conjugations permit the control of the biodistribution, to prolong the blood retention time and to vary the membrane permeability [2-5, 27-29, 35-48]. This development opens a new qualitative application: the resulting complex can be a marker and also a transporter of the active principle to the target.

Other paramagnetic complexes composed by Transition or Lanthanides metals ions are being used in the research, pre-clinical and clinical studies [1-4, 49, 50]. The most successful and promising of these CAs are those with Mn^{n+} complexes.

In the last ten years the attention to MNP as a relative well known development associated to Theranostics has increased [1-4, 27, 30, 51-59]. The Iron core in this MNP is coupled with a biopolymer in order to make the particle biocompatible. This biopolymer around the core allows also the conjugation of contrast and therapeutic agents. Recently, the biodistribution and pharmacokinetics of uniform magnetite MNP chemically-modified with polyethylene glycol has been studied [60]. By successive MRI, the evolution of tissue contrast, absorption speed, time of residence and excretion of this MNP was followed in the liver for a month. On the basis of these results, different metabolic routes that determine the fate of MNP has been proposed [60].

The carbon nanotubes and other nanostructures like nanobubbles in colloid suspension are other flexible nanoframeworks for the mMRI [2-4, 61-64]. They have a very large free surface in which is possible to coordinate different types of drugs, monoclonal antibodies, CA, cell penetration peptides and molecules to the control of the drug delivery and release [1-5, 61-64].

The diversity of possible molecular arrangements is practically infinite; nevertheless, the most important are those which have a flexible framework, for example, the dendrimers and the PEG [2-4, 21, 27, 50, 52, 65-71]. These two macromolecular platforms permit forming a very large specific molecular formation designed to solve great concrete biopharmaceutical problems [2-4, 39-42, 65-69, 72]. These macromolecular formation are flexible because is possible to control the molecular mass, size and shape with a relative high precision. Also, it has a relative high number of functional group (reactive sites) to be conjugated with CA, solubility improver, receptor ligand and drugs. In the case of PEG it is also possible to change the core size [69].

It has been demonstrated that the size control is determinant to improve, besides other properties, the relativities, blood retention time and also influences targeting of the Theranostics formulation [3, 4, 6, 27, 39-48, 65-71, 73, 74]. A dendrimer conjugated with Gd(DTPA) called Gadomer 17 have a relaxivity 16 $mM^{-1}s^{-1}$, *i.e.*, about four times greater than the Gd(DTPA) [65]. In another report [66] these nanoparticles can be preferentially targeted to blood or to the lymphatic track depending on the particle's size. The lowest the size of the dendrimers, the highest the distribution into the blood vessels, with dendrimers being increasingly distributed into the lymphatic vessels at larger particle sizes. Some groups have evaluated the rheology, permeability and other properties of these NP formulations as a function of particles' shape [73, 74].

The dendrimer and PEG flexibility provides a tight control over the absorption, the distribution and clearance profiles of the formulations based on these polymers. That's why they have been explored as delivery vectors for the different drug administration routes: oral, intravenous, subcutaneous, pulmonary and even transdermal delivery [66].

Multifunctional, multimodal and multistage nanoprobes combining CA for different imaging modalities (for example PET and MRI) either the arrangement of different active molecules for the combined therapies have attracted great interest [75-79].

Much more extended studies have been carried out using macromolecular formations such as: liposomes, ensomes, miscelle ensome, mesomes and LipoCEST [2-4, 33, 80-82]. Some of these molecular formations are self-assembly NP. They are generally composed of copolymers with both hydrophobic and hydrophilic segments. The core of the NP and its surface provide an effective loading compartment for the drug and the CA. Nevertheless, some of these molecular structures have a relatively low relaxivity because of the small intensity water exchange among particle associated water and the free one.

Natural macromolecular formations akin to high density lipoproteins (HDL) [2-4, 83-86], Ferritin and virus-like particles (VLPs) [83] have been conjugated with different paramagnetic complexes, drugs and molecular improver. The obtained particles have very good Theranostics characteristics including a high relaxivity and the capacity to be loaded with elevated amounts of paramagnetic complexes. These natural macromolecular particles have several advantages because of their relatively low toxicity, good pharmacological behavior and also because the immunological system does not reject it [83]. In an early work [87] human and rat red blood cells were loaded with Gd(DTPA) to create a blood pool MRI CA. The relaxivity tended to increase, together with retention times and contrast enhancement in different organs.

A family of biodegradable macromolecular CAs has also been reported [2-4, 43-46]. Some of these CAs are based on proteins polymers allowing control over CA properties, as well as the polydisulfide Gd complexes [45, 46]. These novel formations can act as macromolecular CA for *in vivo* imaging and be excreted rapidly as a low molecular weight agent. The progress in the area of biodegradable CAs is very promising. In summary, these nanoparticulated macromolecular formations mentioned provide a promising platform for Theranostic formulation of increased delivery and release control, optimizing the residence time and the permeability in the different compartments, improving drug targeting and CA loading into the target, decreasing the toxicity and increasing the relaxivity, and, as consequence, the sensitivity of the mMRI.

32. Hylton N. Dynamic contrast-enhanced magnetic resonance imaging as an imaging biomarker. J Clin Oncol. 2006; 24:3293-8.

33. Winter PM. Magnetic resonance chemical exchange saturation transfer imaging and nanotechnology. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012;4:389-98.

34. Caravan P, Zhang Z. Structure-relaxivity relationships among targeted MR contrast agents. Eur J Inorg Chem. 2012; 2012(12):1916-23.

35. Zong Y, Wang X, Goodrich KC, Mohs AM, Parker DL, Lu ZR. Contrast-enhanced MRI with new biodegradable macromolecular Gd(III) complexes in tumor-bearing mice. Magn Reson Med. 2005;53:835-42.

36. Chan KW, Wong WT. Small molecular gadolinium(III) complexes as MRI contrast agents for diagnostic imaging. Coord Chem Rev. 2007;251:2428-51.

37. Meade TJ, Taylor AK, Bull SR. New magnetic resonance contrast agents as biochemical reporters. Curr Opin Neurobiol. 2003;13:1-6.

38. Jasanoff A. Functional MRI using molecular imaging agents. Trends Neurosci. 2005;28:120-6.

39. Caravan P. Protein-targeted gadolinium-based magnetic resonance imaging (MRI) contrast agents: design and mechanism of action. Acc Chem Res. 2009; 42:851-62.

40. Shahbazi-Gahrouei D, Roufeh M, Tavakoli MB. Gadolinium-Diethylenetriaminepenta-Acetic acid Conjugated with Monoclonal Antibody C595 as New Magnetic Resonance Imaging Contrast Agents for Breast Cancer (MCF-7) Detection. Iranian Biomed J. 2006;10:109-213.

41. Chen Z, Yu D, Liu C, Yang X, Zhang N, Ma C, *et al*. Gadolinium-conjugated PLA-PEG nanoparticles as liver targeted molecular MRI contrast agent. J Drug Target. 2011;19:657-65.

42. Mohs AM, Lu ZR. Gadolinium(III)-based blood-pool contrast agents for magnetic resonance imaging: status and clinical potential. Expert Opin Drug Deliv. 2007;4:149-64.

43. Mohs AM, Wang XH, Goodrich KC, Zong YD, Parker DL, Lu ZR. PEG-g-poly (GdDTPA-co-L-cystine): a biodegradable macromolecular blood pool contrast agent for MR imaging. Bioconjugate Chem. 2004;15:1424-30.

44. Lu ZR, Ye F, Vaidya A. Polymer platforms for drug delivery and biomedical imaging. J Control Release. 2007;122:269-77.

45. Lu ZR, Mohs AM, Zong Y, Feng Y. Polydisulfide Gd(III) chelates as biodegradable macromolecular magnetic resonance imaging contrast agents. Int J Nanomed. 2006;1:31-40.

46. Lu ZR, Parker DL, Goodrich KC, Wang X, Dalle JG, Buswell HR. Extracellular biodegradable macromolecular gadolinium(III) complexes for MRI. Magn Reson Med. 2004;51:27-34.

47. Chen KJ, Wolahan SM, Wang H, Hsu CH, Chang HW, Durazo A, *et al*. A small MRI contrast agent library of gadolinium(III)-encapsulated supramolecular nanoparticles for improved relaxivity and sensitivity. Biomaterials. 2011; 32:2160-5.

The conjugation of paramagnetic complexes with versatile (flexible) macromolecules and natural nanostructures is the most important and fastest way to obtain new Theranostic formulations at present.

Despite the faster growth in number of Theranostic nanocarriers in latest years, the number of these formulations approved for clinical purposes is still very few and its relativities are in a relative low narrow range [4, 88]. Nevertheless, a growing rate of approved compounds for clinical use is expected to be increased in the near future.

New Theranostics nanocarriers with a very high relaxivity implying an essential raise of the mMRI sensibility were announced a few years ago. As example [4, 89, 90], a dendrimer was presented as component of a new porous carrier system. This porous carrier system has three key component features: a stabilized polymer shell, a porous membrane structure and dendrimer-attached Gd complexes encapsulated inside the porous vesicular carrier system. This macronano structure has a relaxivity greater than 300 000 $mM^{-1}s^{-1}$. Another interesting nanocarrier was developed [59], in which the obtained NP based on perfluorocarbon carrying 94 000 Gadolinium chelates produced relativities of 1.69×10^6 $mM^{-1}s^{-1}$ at 1.5 T and 910 000 $mM^{-1}s^{-1}$ at 4.7 T. Strikingly, this is approximately one million times greater than Gd (DTPA). This enables a minimum detection limit (sensitivity) in the range of a few to 10^2 pmoles concentration [59]. It is predictable that these Theranostics formulations will play a special role in the development of new nanomacromolecular formations and that the sensitivity of mMRI will be increased in several orders of magnitude.

Let´s emphasize that the mMRI sensitivity increases as a result of the development of new kind of Theranostics formulations, in several order higher than those obtained by the high cost MRI hardware progress. The increase in sensitivity will allow to widen the range of application and to extend the use of clinical MRI equipments to the pre-clinical studies progressively.

Some remarks, challenges, tasks and expected output

To successfully exploit the opportunities of Molecular Imaging for drug development, several challenges and tasks need to be taken into account. Let's mention some of them.

A very important restriction of the Theranostics impact is associated with the development and validation of custom-made Theranostics new formulations. Theranostics formulations for the clinical studies must move throughout the sequences of steps for drug discovery and development until its approval by a regulatory agency. However, it is possible to design strategies to accelerate this process with an optimum selection of the molecular framework. Whereas the process is occurring, it's possible and necessary to increment the intensity and effectiveness use of the Theranostic formulations in the research and the preclinical studies.

As a result of new hardware and software development either of the existing methods and novel principles, it is possible to affirm that new imaging technologies will be introduced in the near future. Effective efforts should be done to decrease the cost of MRI machines, which limits its extended use.

Only some words to pay our attention to a very new imaging method: the Magnetic Particle Imaging, which emerged few years ago [91-93]. This new technology together with the MRI promises to have a very high sensitivity and resolution in the same machine [93].

Nevertheless, the most important expected improvement will occur in the field of original Theranostics biodegradable formulation, decrease of toxicity and enhancement of the delivery, release and clarifying profiles. Simultaneously, this formulation will enlarge the efficacy of the combined and personalized therapies and its imaging control.

More effective and standardized methods for the characterization of the molecular formation used in the Theranostics are emerging. The understanding of the molecular interaction inside the living bodies with the CA is essential for these new developments.

Delivering the drug to the target with high precision and control is the essential endeavor of the drug delivery systems. This growing technology represents one of the most rapidly advancing areas; and specially, controlled drug release as a field of pharmaceutical technology has rapidly diversified recently [94].

Responsive drug delivery and releasing systems have essentially two main parts: a sensor that detects the environmental parameter which stimulates drug release and the respective delivery device [94]. Signals that have been employed to trigger drug release can be classified into two groups: those who are intrinsic and those who are extrinsic to the body, and according to a definite physiological characteristic or external radiation triggered the release of the drugs contained within a specific nanocarrier.

In the Theranostics approach, several terms have been coined for carriers, such as 'intelligent' or 'smart' carriers, because of their ability to manage a stimulus by receiving, transmitting and processing it; and also, they are 'intelligent' in the sense that they can perceive the prevailing stimuli and respond by exhibiting changes in their physical or chemical behavior, resulting in a controlled release of entrapped or attached drug or molecular improvers such as permeability and retention time [95]. Among the intrinsic we can find: pH mechanism [23, 95-98], temperature sensitive [23, 99], enzymatic stimuli reactive [96-98], diffusion controlled [95, 96], antibodies targeted [23, 85] and redox potential [97]. The extrinsic include: nanoelectromechanical systems [100-102], magnetic field [61, 97, 100, 103], electric field, ultrasonic signals [100], light and laser exposure [97, 100, 104-107], and others. The development in this ground is really starting; however, a relative not easy way is necessary to be crossed to make all those advances compatible with the concrete biological, biomedical and biopharmaceutical requirements and regulations. Molecular Imaging technologies can make a contribution in this direction.

Lastly, in order to make an optimum use and to contribute toward the enlargement of the possibilities of Molecular Imaging is important to have the best design of the pre-clinical and clinical trials. Imaging methods must be taken into account from the very

48. Karfeld-Sulzer LS, Waters EA, Davis NE, Meade TJ, Barron AE. Multivalent protein polymer MRI contrast agents: controlling relaxivity via modulation of amino acid sequence. Biomacromolecules. 2010;11:1429-36.

49. Pan D, Caruthers SD, Senpan AF, Schmieder AH, Wickline SA, Lanza GM. Revisiting an old friend: manganese-based MRI contrast agents. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2010;3.

50. Zhen Z, Xie J. Development of manganese-based nanoparticles as contrast probes for magnetic resonance imaging. Theranostics. 2012;2:45-54.

51. Tong S, Hou S, Zheng Z, Zhou J, Bao G. Coating optimization of superparamagnetic iron oxide nanoparticles for high T2 relaxivity. Nano Lett. 2010;10:4607-13.

52. Veiseh O, Gunn JW, Zhang M. Design and fabrication of magnetic nanoparticles for targeted drug delivery and imaging. Adv Drug Deliv Rev. 2010;62:284-304.

53. Colombo M, Carregal-Romero S, Casula MF, Gutierrez L, Morales MP, Bohm IB, *et al*. Biological applications of magnetic nanoparticles. Chem Soc Rev. 2012;41:4306-34.

54. Xie J, Jon S. Magnetic nanoparticle-based theranostics. Theranostics. 2012; 2:122-4.

55. Shao H, Min C, Issadore D, Liong M, Yoon TJ, Weissleder R, *et al*. Magnetic Nanoparticles and microNMR for Diagnostic Applications. Theranostics. 2012;2:55-65.

56. Shubayev VI, Pisanic TR, Jin S. Magnetic nanoparticles for theragnostics. Adv Drug Deliv Rev. 2009;61:467-77.

57. Rumenapp C, Gleich B, Haase A. Magnetic nanoparticles in magnetic resonance imaging and diagnostics. Pharm Res. 2012;29:1165-79.

58. Prow TW, Grice JE, Lin LL, Faye R, Butler M, Becker W, *et al*. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev. 2011;63:470-91.

59. Morawski AM, Winter PM, Crowder KC, Caruthers SD, Fuhrhop RW, Scott MJ, *et al*. Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI. Magn Reson Med. 2004;51:480-6.

60. Ruiz A, Hernandez Y, Cabal C, Gonzalez E, Veintemillas-Verdaguer S, Martinez E, et al. Biodistribution and pharmacokinetics of uniform magnetite nanoparticles chemically modified with polyethylene glycol. Nanoscale. 2013 Jun 10. doi: 10.1039/C3NR01412F.

61. Yoo D, Lee JH, Shin TH, Cheon J. Theranostic magnetic nanoparticles. Acc Chem Res. 2011;44:863-74.

62. Jokerst JV, Gambhir SS. Molecular imaging with theranostic nanoparticles. Acc Chem Res. 2011;44:1050-60.

63. Liu Z, Liang XJ. Nano-carbons as theranostics. Theranostics. 2012;2:235-7.

64. Chen Z, Ma L, Liu Y, Chen C. Applications of functionalized fullerenes in tumor theranostics. Theranostics. 2012; 2:238-50.

65. Nicolle GM, Toth E, Schmitt-Willich H, Raduchel B, Merbach AE. The impact of rigidity and water exchange on the relaxivity of a dendritic MRI contrast agent. Chemistry. 2002;8:1040-8.

beginning. Before starting the trials, it is necessary to consider the standardization and the synchronization of the diagnostic and therapy protocols. In the Theranostics approach this complex problem may find an outstanding solution**.**

In summary, mMRI techniques are increasing the contrast, resolution (below 50 μm), sensitivity (in the range of nano to some picomoles) and creating new and powerful tools to make more and more important contributions to research and development in biology, biomedicine, and either the biopharmaceutical and biotechnological industries. In fact, Molecular Imaging is becoming the largely expected tool that could contribute to decrease time, costs and to raise the robustness of the stages for drug discovery and development.

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66. Kaminskas LM, Porter CJ. Targeting the lymphatics using dendritic polymers (dendrimers). Adv Drug Deliv Rev. 2011; 63:890-900.

67. Bumb A, Brechbiel MW, Choyke P. Macromolecular and dendrimer-based magnetic resonance contrast agents. Acta Radiol. 2010;51:751-67.

68. Kobayashi H, Kawamoto S, Saga T, Sato N, Hiraga A, Ishimori T, *et al*. Positive effects of polyethylene glycol conjugation to generation-4 polyamidoamine dendrimers as macromolecular MR contrast agents. Magn Reson Med. 2001;46:781-8.

69. Cyran CC, Fu Y, Raatschen HJ, Rogut V, Chaopathomkul B, Shames DM, *et al*. New macromolecular polymeric MRI contrast agents for application in the differentiation of cancer from benign soft tissues. J Magn Reson Imaging. 2008;27:581-9.

70. Zhu D, Lu X, Hardy PA, Leggas M, Jay M. Nanotemplate-engineered nanoparticles containing gadolinium for magnetic resonance imaging of tumors. Invest Radiol. 2008; 43:129-40.

71. Nune SK, Gunda P, Majeti BK, Thallapally PK, Forrest ML. Advances in lymphatic imaging and drug delivery. Adv Drug Deliv Rev. 2011; 63:876-85.

72. Bawarski WE, Chidlowsky E, Bharali DJ, Mousa SA. Emerging nanopharmaceuticals. Nanomedicine. 2008;4:273-82.

73. Ulbrich W, Lamprecht A. Targeted drug-delivery approaches by nanoparticulate carriers in the therapy of inflammatory diseases. J R Soc Interface. 2010;7 Suppl 1:S55-S66.

74. Venkataraman S, Hedrick JL, Ong ZY, Yang C, Ee PL, Hammond PT, *et al*. The effects of polymeric nanostructure shape on drug delivery. Adv Drug Deliv Rev. 2011;63:1228-46.

75. McCarthy JR. Multifunctional agents for concurrent imaging and therapy in cardiovascular disease. Adv Drug Deliv Rev. 2010;62: 1023-30.

76. Louie A. Multimodality imaging probes: design and challenges. Chem Rev. 2010;110: 3146-95.

77. Godin B, Tasciotti E, Liu X, Serda RE, Ferrari M. Multistage nanovectors: from concept to novel imaging contrast agents and therapeutics. Acc Chem Res. 2011;44:979-89.

78. Yu MK, Park J, Jon S. Targeting strategies for multifunctional nanoparticles in cancer imaging and therapy. Theranostics. 2012; 2:3-44.

79. De M, Chou SS, Joshi HM, Dravid VP. Hybrid magnetic nanostructures (MNS) for magnetic resonance imaging applications. Adv Drug Deliv Rev. 2011;63:1282-99.

80. Kozlowska D, Foran P, MacMahon P, Shelly MJ, Eustace S, O'Kennedy R. Molecular and magnetic resonance imaging: The value of immunoliposomes. Adv Drug Deliv Rev. 2009; 61:1402-11.

81. Shi J, Xiao Z, Kamaly N, Farokhzad OC. Self-assembled targeted nanoparticles: evolution of technologies and bench to bedside translation. Acc Chem Res. 2011;44: 1123-34.

82. Al-Jamal WT, Kostarelos K. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for theranostic nanomedicine. Acc Chem Res 2011; 44:1094-104.

83. Cormode DP, Jarzyna PA, Mulder WJ, Fayad ZA. Modified natural nanoparticles as contrast agents for medical imaging. Adv Drug Deliv Rev. 2010;62:329-38.

84. Felber AE, Dufresne MH, Leroux JC. pH-sensitive vesicles, polymeric micelles, and nanospheres prepared with polycarboxylates. Adv Drug Deliv Rev. 2012;64:979-92.

85. Nystrom AM, Wooley KL. The importance of chemistry in creating well-defined nanoscopic embedded therapeutics: devices capable of the dual functions of imaging and therapy. Acc Chem Res. 2011;44:969-78.

86. Ng KK, Lovell JF, Zheng G. Lipoprotein-inspired nanoparticles for cancer theranostics. Acc Chem Res. 2011;44:1105-13.

87. Johnson KM, Tao JZ, Kennan RP, Gore JC. Gadolinium-bearing red cells as blood pool MRI contrast agents. Magn Reson Med. 1998;40:133-42.

88. Wong FC, Kim EE. A review of molecular imaging studies reaching the clinical stage. Eur J Radiol. 2009;70:205-11.

89. Cheng Z, Tsourkas A. Paramagnetic porous polymersomes. Langmuir. 2008;24:8169-73.

90. Mody VV, Nounou MI, Bikram M. Novel nanomedicine-based MRI contrast agents for gynecological malignancies. Adv Drug Deliv Rev. 2009;61:795-807.

91. Weizenecker J, Gleich B, Rahmer J, Dahnke H, Borgert J. Three-dimensional real-time *in vivo* magnetic particle imaging. Phys Med Biol. 2009;54:L1-L10.

92. Weizenecker J, Borgert J, Gleich B. A simulation study on the resolution and sensitivity of magnetic particle imaging. Phys Med Biol. 2007;52:6363-74.

93. Udrea LE, Strachan NJ, Badescu V, Rotariu O. An *in vitro* study of magnetic particle targeting in small blood vessels. Phys Med Biol. 2006;51:4869-81.

94. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev. 1999;99: 3181-98.

95. Gupta P, Vermani K, Garg S. Hydrogels: from controlled release to pH-responsive drug delivery. Drug Discov Today. 2002;7:569-79.

96. Kim S, Kim JH, Kim D. pH sensitive swelling and releasing behavior of nano-gels based on polyaspartamide graft copolymers. J Colloid Interface Sci. 2011;356:100-6.

97. Ambrogio MW, Thomas CR, Zhao YL, Zink JI, Stoddart JF. Mechanized silica nanoparticles: a new frontier in theranostic nanomedicine. Acc Chem Res. 2011;44:903-13.

98. Koo H, Huh MS, Sun IC, Yuk SH, Choi K, Kim K, *et al*. *In vivo* targeted delivery of nanoparticles for theranosis. Acc Chem Res. 2011;44:1018-28.

99. Yu SS, Scherer RL, Ortega RA, Bell CS, O'Neil CP, Hubbell JA, *et al*. Enzymatic- and temperature-sensitive controlled release of ultrasmall superparamagnetic iron oxides (USPIOs). J Nanobiotechnol. 2011;9:7.

100. LaVan DA, McGuire T, Langer R. Small-scale systems for *in vivo* drug delivery. Nat Biotechnol. 2003;21:1184-91.

101. Staples M. Microchips and controlled-release drug reservoirs. WIREs Nanomed Nanobiotechnol. 2012;2:400-17.

102. Sharma S, Nijdam AJ, Sinha PM, Walczak RJ, Liu X, Cheng MM, *et al*. Controlled-release microchips. Expert Opin Drug Deliv. 2006;3:379-94.

103. Namiki Y, Fuchigami T, Tada N, Kawamura R, Matsunuma S, Kitamoto Y, *et al*. Nanomedicine for cancer: lipid-based nanostructures for drug delivery and monitoring. Acc Chem Res. 2011;44:1080-93.

104. Melancon MP, Zhou M, Li C. Cancer theranostics with near-infrared light-activatable multimodal nanoparticles. Acc Chem Res. 2011;44:947-56.

105. Puri A, Blumenthal R. Polymeric lipid assemblies as novel theranostic tools. Acc Chem Res. 2011;44:1071-9.

106. Bardhan R, Lal S, Joshi A, Halas NJ. Theranostic nanoshells: from probe design to imaging and treatment of cancer. Acc Chem Res. 2011;44:936-46.

107. Xia Y, Li W, Cobley CM, Chen J, Xia X, Zhang Q, *et al*. Gold nanocages: from synthesis to theranostic applications. Acc Chem Res. 2011;44:914-24.

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