METRICS project: Synthesis of Manganese Complexes as potential PET/MRI Agents

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INTRODUCTION

Molecular imaging (MI) is diagnostic technique allowing for the visualization, characterization and measurement of biochemical process at the molecular and cellular level in living organisms. MI has received considerable interest during the last few decades because of its momentous potential in diagnosis and treatment monitoring for both preclinical and clinical applications; MI includes several techniques that can give either functional information, such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), and anatomical information, such as magnetic resonance imaging (MRI) or computed tomography (CT). One single imaging modality may not be sufficient to obtain all necessary information and each single MI technique has its own strengths and drawbacks. For example, PET and SPECT, which have been used extensively to visualize metabolic and physiological process, offer very high sensitivity (in the pico-nano-molar range) but provide relatively poor anatomic resolution. On the other hand, primarily anatomical imaging modalities such as MRI and CT have been used to detect anatomical structures because of their high spatial resolution, but with relatively poor sensitivity (in the millimolar range).
Thus, there is an increasing interest in the combination of two or more imaging modalities that can both provide complementary information regarding morphology and functioning and achieve synergistic advantages over individual modes. It is known as Multi-Modality Imaging (MMI) technique [1]. These technologies are already consolidated and it is interesting to note that they allow the superimposition of images recorded with PET or SPECT, which are then fused with CT or MRI images.
Moreover, the contrast agent for MRI and the radioactive labelled compound for PET, are of different chemical species that gives rise to an inescapable mismatch about the content of the diagnostic information. The only way to achieve a genuine molecular fusion between PET and MRI is to use chemically identical contrast and radioactive probe. To overcome such an issue, METRICS project is born with the aim to produce Mn-51 and Mn-52 with cyclotron and also to develop stable paramagnetic Mn(II) complexes for multimodal PET/MRI imaging.
The transition element manganese was selected because has stable isotopes with useful magnetic properties to allow MRI and a couple of positron-emitting radioisotopes, Mn-52 and Mn-51, that could be employed as PET tracers.
Manganese, however, in its free form is neurotoxic (LD50 = 0,22 mmol/kg for rat) [2-3] and therefore, in order to prevent the premature release of the metal and ensure in vivo safety, the Mn^{2+} ion needs to be chelated by ligands to form stable Mn-complexes..
Recently, in the framework of METRICS project, several manganese-based complexes (focused on paramagnetic complexes Mn^{2+}), using different coordinating atoms, have been synthesized and characterized. In vitro stability studies were also performed.

EXPERIMENTAL

We have investigated the reactivity of the manganese ion (Mn^{2+}), as Mn-chloride and Mn-acetate, against ligands with different coordinating atoms, in particular:
- bidentate ligands having a set of S-S and N-S coordinating atoms;
- tetradentate ligands with N_2S_2 donor set;
- bidentate monoanionic ligands O-O and
- neutral ligands.

The coordination reactions of the selected ligands with the metal salts were carried out in one single step . A solution of the manganese salt in different solvents (water, methanol, etc...) was added to the ligand solubilized with the same solvent in stoichiometric ratio 1:2 or, in some cases, 1:3. The product, which immediately precipitate, was: separated from the solution, washed first with the reaction solvent and then with diethyl ether or ethanol-diethyl ether (2:1) and finally allowed to dry in vacuum, fig.1. With the aim to obtain crystalline solids for further characterization of the products, crystallization tests with different solvents, are in progress.

Fig.1. Pictures of the synthesis and filtration steps.

Moreover, the $Mn(PTA)_2(H_2O)_2Cl_2$ (PTA=1,3,5-triaza-7-phosphaadamantane, Fig.2) complex was also synthetized [4] and used to assess its reactivity with the above listed ligands.

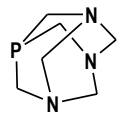

Fig.2. PTA=1,3,5-triaza-7-phosphaadamantane

These reactions were carried out in two steps and involved first, the reaction of the metal salts with the PTA, followed by the addition of the selected ligand. A solution of the Mn salt in methanol was added to the PTA ligand, solubilized with methanol in stoichiometric ratio 1:2. The product, which immediately precipitate from the solution, was filtrated, washed first with methanol and then with diethyl ether and finally allowed to dry in vacuum. The selected bidentate or tetradentate ligand, solubilized in water, was mixed with the $Mn(PTA)_2$ solution to form the final product that was separated and washed first with water and then with diethyl ether and finally dried in vacuum.
Crystallization tests are also in progress.
All the complexes were characterized by:
- IR analysis,
- elemental analysis (EA),
- cyclic voltammetry,
- HPLC analysis.
Finally, to assess the stability of the complex, serum stability studies on selected complexes have been carried out, by HPLC.
A summary of the synthesized and characterized complexes using different analysis techniques is reported in table 1.

Table 1. Ligands used for the synthesis of manganese-based complexes

Ligand
oxalate
2-thiopyridine
diethyldithiocarbamate
Betaine
L-Carnitine
PTA

RESULT AND DISCUSSION

Manganese is an attractive multimodal PET/MRI imaging agent but is not free from toxicity and therefore, in order to ensure in vivo safety, the Mn^{2+} ion needs to be chelated by ligands that provide high stability to the complex, thus preventing the release of the free metal. With this aim in mind, manganese-based agents have been prepared evaluating different coordinating systems for establishing trends of how the ligand structure and the rigidity of the ligand scaffold influence the stability of the complex. All the reactions were performed starting from $MnCl_2$ and $Mn(CH_3CO_2)_2$ reagents and complexes of the $Mn(II/III)(O\sim O)_n$, $Mn(II)(S_2N_2)$, $Mn(II/III)(S\sim S)_n$, $Mn(N\sim S)_n$ (n=2,3) type have prepared in yield >60% .
In vitro serum stability studies, performed on selected complexes have highlighted that the metabolism is rapid and only after 5 min of incubation the Mn-complex is no longer stable; this is in agreement with the behavior of the Mangafodipir, a commercial contrast agent for magnetic resonance imaging, that, however, shows a slower metabolism in vivo compared with in vitro studies [5].

CONCLUSION

This work describes the syntheses, characterizations and in vitro stability studies performed so far within the METRICS project on the production of stable paramagnetic Mn isotopes for multimodal PET/MRI imaging.
Some complexes have been selected as hopeful candidates for further in imaging studies.

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REFERENCES AND FINAL NOTES

[1] L.Martí-Bonmatí et al. Contrast Media and Mol. Imaging, 2010, vol. 5, issue 4, 180-189
[2] M. Aschner, K. M. Erikson, D.C. Dorman, Crit. Rev. Toxicol. 2005, 35, 1-32
[3] A. C. Silva, J. H. Lee, I. Aoki, A. P. Koretsky, NMR Biomed. 2004, 17, 532-543
[4] A. C. Ekennia et al., Bioinorganic Chemistry and Applications, 2015
[5] K. Gunnar et al., J. of Pharmaceutical and Biomedical analysis, 1997, 15, 983–988