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SCIENTIFIC REVIEWS

Importance of Radiopharmacy in Hospital Practice: Application to Alzheimer and Parkinson's Disease Exploration

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Summary

The purpose of this work is to describe the importance of radiopharmacy in hospital practice in relation with the nuclear medicine activity by giving an overview of its application and of relevant rules concerning the preparation and quality control of radiopharmaceutical compounds. The importance will be illustrated by the molecular imaging exploration of Alzheimer and Parkinson's diseases.
Key Words: *Radiopharmaceuticals, radiopharmacy, quality requirements, neurodegenerative diseases.*
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INTRODUCTION

In Europe, radiopharmaceutical compounds are considered a special group of medicines. Therefore, their preparation and use are regulated by a number of European Union (EU) directives, regulations and rules that have been adopted by member states. These radiopharmaceuticals are becoming very important tools for molecular imaging, which is a new exciting path for nuclear medicine. Indeed, molecular imaging is a non-invasive assessment, visualization, characterization and quantification of gene and protein function, protein-protein interaction, and profiling of signal transduction pathways in patients to obtain further insight into the molecular pathology of a specific disease. The novelty of molecular imaging is that the cellular and molecular pathways and in vivo mechanisms of disease can be directly assessed in a

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physiologically authentic environment on a dynamic and repeated basis[1,2]. Molecular imaging employs probes such as radiopharmaceuticals, which are specific for a certain molecular event, and provides the basis for earlier detection and characterization of disease and therapy evaluation. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are the main modalities to perform this molecular imaging in humans in research and clinical practice modalities using radiopharmaceuticals.

Radiopharmaceutical preparation

Different possibilities exist to provide radiopharmaceuticals to nuclear medicine centers:

1- Licensed radiopharmaceutical products with marketing authorization (MA);
2- Radiopharmaceuticals outside MA, having established clinical use, that are prepared in accordance with approved regulations and meet approved quality requirements (e.g. as described in a monograph of a pharmacopoeia), called magistral preparation and hospital preparation;
3- New products for clinical research.

In each case, these preparations have to be performed

1- under the responsibility of a qualified person who is typically a licensed pharmacist, has several years experience working in pharmaceutical manufacturing operations, and has passed examinations attesting to his or her knowledge.

2- with premises and equipment located, designed, constructed, adapted and maintained to suit the operations to be carried out. Their layout and design must aim to minimize the risk of errors and allow effective cleaning and maintenance in order to avoid cross-contamination, build-up of dust or dirt and, in general, any adverse effect on the quality of the products.

3- and with respect to the rules for radiopharmaceutical preparation as described in the Guidelines on Radiopharmaceutical EMEA/CHMP /QWP / 306970 /2007[3] and Guidelines on Good Radiopharmacy Practice (GRPP) issued by the Radiopharmacy Committee of EANM [4].

Therefore, the role of the radiopharmacist is fundamental, and he has to take charge of the

1. Procurement
2. Preparation
3. Quality Assurance
4. Dispensing
5. Distribution
6. Health and Safety
7. Provision of Information and Consultation
8. Monitoring of Patient Outcome
9. Research and Development

Applications

The strength of imaging techniques for early diagnosis is based on the fact that, as illustrated in Figure 1, significant modifications (increase or decrease) of molecular targets (solid line) occur before the appearance of clinical signs (dotted line in figure). It would constitute a major progress if diagnosis of neurodegenerative diseases could be made prior to the appearance of clinical symptoms (i.e. in the period marked by the green rectangle in the figure) (DiMI program WP3 Development of radiopharmaceutical probes for neurodegenerative diseases; Leader D Guilloteau)[5].

Natural History of Neurodegenerative Disorders (modified from DeKosky & Marek. *Science* 2003. 302:830-834)

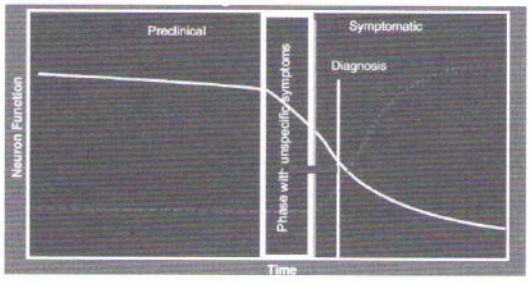

Figure 1 : Natural history of neurodegenerative disorders.

Molecular imaging also enables the optimization of drug therapy by imaging the drug effects at the molecular and cellular level as well as by the assessment of disease progression with and without therapy.

The first step in molecular imaging is to choose the relevant molecular target to explore the disease. A number of molecular targets such as receptors, transporters or enzymes are known to be involved at early stages of these diseases. We can illustrate the role played by molecular imaging with new radiopharmaceuticals in the two main neurodegenerative diseases, Parkinson and Alzheimer diseases.

1-Parkinson Disease

Parkinson disease (PD) is a progressive disabling neurodegenerative disorder. One of the principal anatomopathological characteristics of PD is the loss of dopaminergic neurons. Scintigraphic exploration of the dopamine system using specific ligands has evolved, showing the usefulness of pre-synaptic transporters or post-synaptic receptors as targets of this system[6] (Fig. 2).

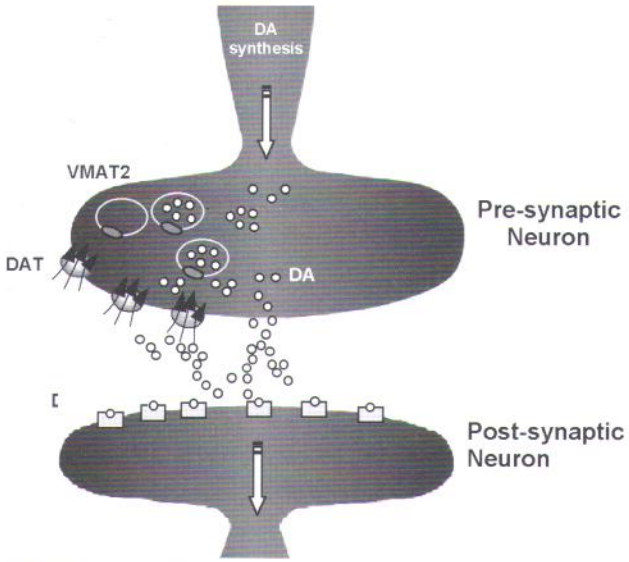

Figure 2 : Molecular targets involved in Parkinson's disease. DA: Dopamine. DAT: Dopamine transporter. D2R: Dopamine D2 receptor. VMAT2: Vesicular monoamine transporter 2.

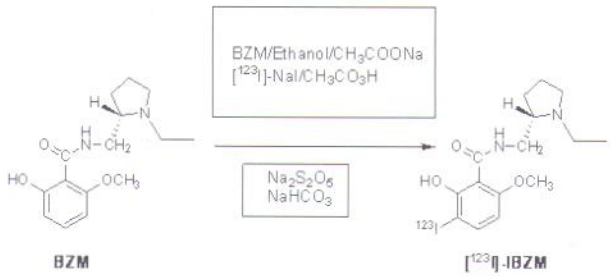

Figure 3 : [^{123}I]-IBZM synthesis.

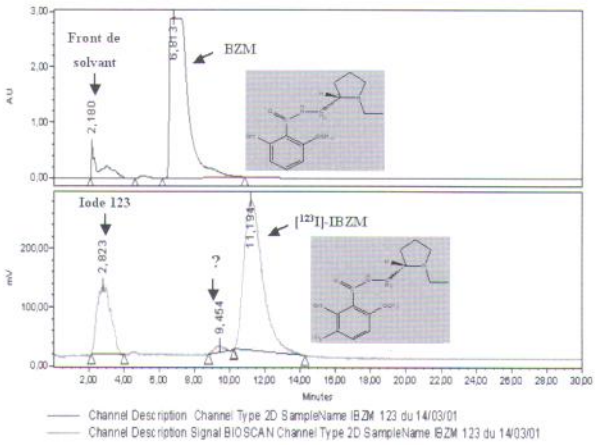

Figure 4 : [^{123}I]-IBZM purification by HPLC.

Pre-synaptic dopamine transporters (DAT):

The first target of the integrity of dopaminergic neurons in PD is the pre-synaptic dopamine transporter (DAT) localized on striatal nerve endings. Many cocaine derivatives have been described in order to visualize this DAT. ^{123}I-FP-CIT (DaTSCAN®) is actually used in Europe with MA to assess the severity of the pre-synaptic dopaminergic lesion[7]. Today, DAT SPECT is mainly used to differentiate between PD and essential tremor[8] (Fig. 5). Here, radiopharmacy is only involved in the dispensing and the quality control of this DAT tracer.

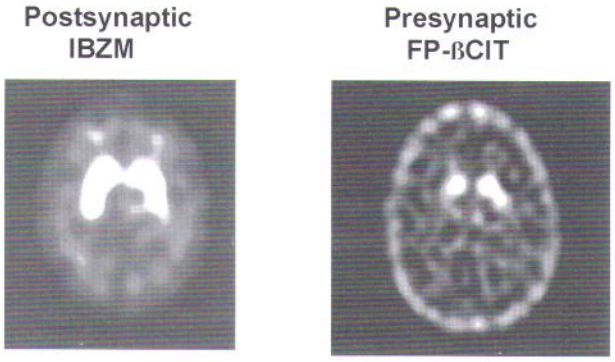

Figure 5 : [^{123}I]-IBZM (post-synaptic) and [^{123}I]-FP-CIT (pre-synaptic) dopaminergic neuro-transmission exploration scans in PD subject. Note the decreased uptake in the pre-synaptic part and normal uptake in post-synaptic part (caudate and putamen).

Dopaminergic post-synaptic D2 receptor:

Dopamine binds mainly to striatal post-synaptic D2 receptors. These receptors in PD are thought to be upregulated in the first years of the disease, especially contralateral to the clinically most affected side. Binding capacity of these receptors can be measured by

SPECT using the D2 radiotracer iodine-123-iodobenzamide ([^{123}I]-IBZM)[6]. This SPECT tracer is a predictor of dopaminergic responsiveness in previously untreated patients with Parkinsonism. This tracer can also be used in the differential diagnosis between PD and other neurodegenerative diseases such as Steele-Richardson-Olszewski disease and multiple system atrophy.

[^{123}I]-IBZM is a magistral preparation. [^{123}I]-IBZM was prepared for human use by oxidative radioiodination of a BZM precursor (Fig. 3). [^{123}I]-IBZM was purified by high pressure liquid chromatography (HPLC) using a RP C^{18} column (Fig. 4). The HPLC fraction containing [^{123}I]-IBZM was loaded onto a Sep-Pack, cartridge, washed with sterile water, and [^{123}I]-IBZM was eluted with 1 ml of 95% ethanol for intravenous injection. The volume of this solution was completed up to 10 ml with 0.9% sodium chloride. Filtration through a 0.22 µm Millipore filter yielded 8-10 ml of sterile and pyrogen-free solution of [^{123}I]-IBZM .

The radiochemical yield following purification was about 60%. Structural identification and radiochemical purity are checked with co-injection of [123I]-IBZM and cold IBZM as reference by HPLC before syringe formulation. This magistral preparation is performed in the radiopharmacy.

2- Alzheimer Disease

Alzheimer's disease (AD) is characterized by a whole of molecular modifications that are either causes or consequences. However, if these modifications are linked to seriousness and/or to the evolution of the disease, structures involved will be potential targets for molecular imaging.

Molecular targets involved in the disease:
The most promising molecular targets are described in Figure 6. AD acts on the cholinergic system and neuro-inflammation/microglial activation with peripheral benzodiazepine receptors (PBR) and amyloid plaques.

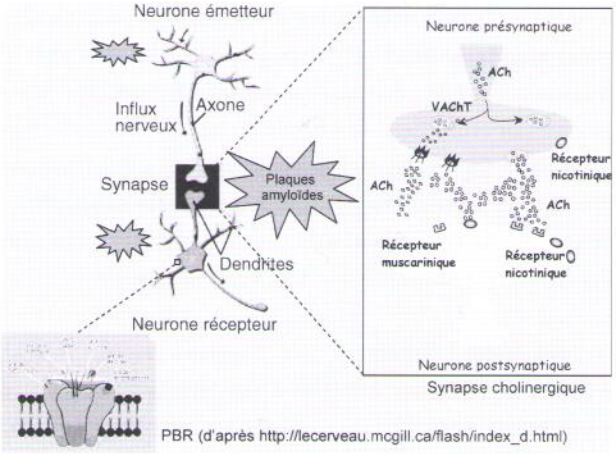

Figure 6 : Molecular targets involved in Alzheimer's disease.

Cholinergic system:
The cholinergic system has huge implications in AD. The number of vesicular acetylcholine transporters (VAChT), localized on the pre-synaptic membrane and responsible for the storage of ACh, decrease during the disease[9]. In vivo, its quantification could be a marker for the diagnosis and follow-up of the disease. Currently, there is a SPECT tracer for the exploration of VAChT: (-)-5-iodobenzovesamicol-iodine 123 ([^{123}I-IBVM). We prepare this radiopharmaceutical for clinical research in our radiopharmacy premises, and have recently demonstrated that a cholinergic degeneration occurs in the early stage of AD and could be involved in the impairment of the cognitive functions[10,11].

The enzyme responsible for the degradation of ACh, the acetylcholinesterase (AChE), is a target for the treatments, the aim of which is to increase the intra-synaptic concentration in ACh.

Visualization and quantification of this AChE are obtainable in PET, by using molecules that are substrates of the enzyme of the type of 11C-MPA4A[12]. Recent results show that the follow-up of treatment with galantamine (inhibiting AChE) can thus be obtained in PET.

Finally, on the post-synaptic membrane, the nicotinic receptor types are also implied in AD. In particular, the number of receptors of the types $\alpha4\beta2$ and $\alpha7$ decrease[13,14]. PET tracers are currently usable under

human investigation or in the course of evaluation for the study of the density of these receptors (ex: 2-fluoro A-85380, derivatives of choline labelled with 11C or 18F, derivatives of epibatidine).

Neuro-inflammation / microglial activation: peripheral benzodiazepine receptors (PBR):

As in most of the neurodegenerative processes, AD is accompanied by neuro-inflammation, which results in a surexpression of PBR. The latter serve as a very interesting molecular target to explore and thus better understand physiopathological mechanisms. This target could also potentially be helpful to follow-up the AD evolution. A PET tracer labelled with carbon-11 (11C-PK11195) (Fig. 7) has been largely used in human imaging; nevertheless, its utility is limited by the carbon-11 (11C) short half-life (20 min) and its high non-specific binding. To overcome these problems, new tracers labelled with fluorine-18 are under validation[15-18].

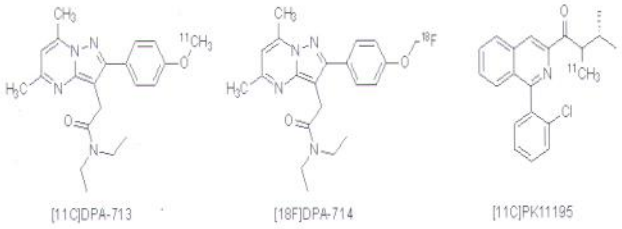

Figure 7: [11C]DPA-713, [18F]DPA-714 and [11C]PK11195 chemical structures; DPA 714 is currently produced in our radiopharmacy for clinical research.

[18F]-DPA is currently produced in our radiopharmacy for clinical research.

Amyloid plaques

The certain diagnosis of AD is highlighted by the post-mortem cerebral examination, and the characteristic lesions of the disease, which include:

- neurofibrillary tangles (NFT)
- accumulation of protein β-amyloid within senile plaques
- neuronal death

Molecular imaging techniques make it possible to consider quantification and localization by visualization of these neuro-pathologic lesions. Many tracers are proposed to visualize the amyloid plaques in vivo, as shown in Figure 8.

To date, the tracer most frequently used is Pittsburgh Compound B-carbon 11 ([11C]-PIB). Several clinical studies have already shown that after cerebral imaging analysis it is possible to clearly separate the healthy control, the mild cognitive impaired (MCI) and the patients with AD[19,20]. The very important question is to distinguish among the MCI those who will develop AD from those who will not. The first studies using PIB have so far yielded promising results[21] (Fig. 8).

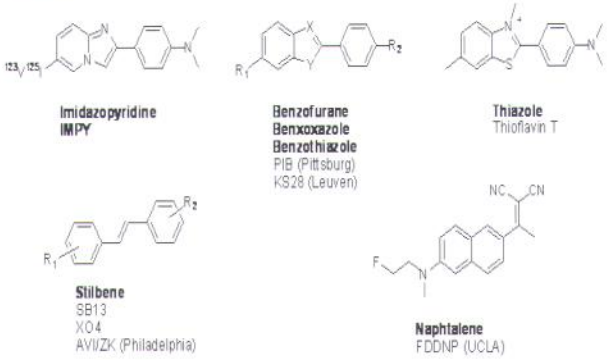

Figure 8: SPECT and PET tracers for amyloid plaque visualization.

As discussed previously, the use of 11C is limited in clinical investigations by its short half-life. Different radiotracers labelled with fluorine-18 have been developed and are under human validation. One of these tracers, the [18F]-FDDNP, has been tested in a study including 25 healthy controls, 28 MCI and 30 AD. The separation of the various categories of healthy controls, MCI and AD seemed possible[22].

Other fluorinated tracers belonging to the family of stilbene (ex: AVI/ZK) are also under validation (Fig. 9). A clinical study carried out in Australia shows very promising results[21,23]. Lastly, a SPECT tracer labelled with iodine 123, the 6-iodo-2- (4' - dimethylamino-) phenyl-imidazo [1,2-a] pyridine (IMPY), was developed by the team of Philadelphia and is under evaluation[24].

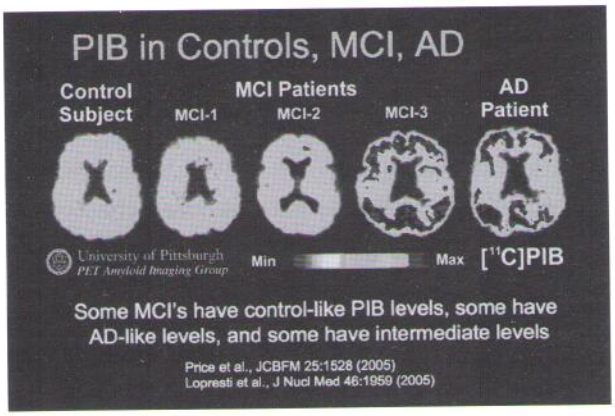

Figure 9: [11C]-PIB–PET scans in control, MCI and AD subjects (with authorization from Dr. Price [Pittsburg]).

CONCLUSION

Radiopharmacy in hospitals is essential in order to prepare and control radiopharmaceuticals. The radiopharmacist can insure the quality of the preparation of MA products, and of magistral and hospital preparations. Moreover, the radiopharmacy can and must take charge of the preparation of products for research and allow the development of new diagnostic methods.

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