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LABELLING OF A NEW SEROTONINERGIC LIGAND : [^{18}F]RITANSERIN

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SUMMARY

Ritanserin has been labelled with ^{18}F to visualize the serotoninergic receptors by Positron Emission Tomography (PET). The synthesis was carried out by a nucleophilic substitution of a nitro substituent of a nitrophenylketone by $^{18}F^-$ and then addition of a Grignard reagent to the ketone followed by dehydration of the tertiary alcohol so formed.

The total time to have [^{18}F]ritanserin ready for injection into a patient was 150 minutes (after the end of bombardement for the ^{18}F production [^{18}O (p,n) ^{18}F]).

The labelling technique described was used to obtain 1.1 to 1.5 GBq (30 to 40 mCi) of [^{18}F]Ritanserin with a specific activity of 44 GBq/μmol (1.2 Ci/μmol) starting from 18.5 GBq (500 mCi) ^{18}F.

Key words : [^{18}F]Ritanserin, Positron Emission Tomography, [^{18}F]Production.

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INTRODUCTION

Among the various classes of serotoninergic binding sites, the serotoninergic S_2-receptor is the best characterized in pharmacological and physiological terms (1) and may be implicated in several neurological as well as mental disorders. The in vivo study of the S_2-receptors in these human conditions would be of great interest.

Using Positron Emission Tomography (PET) several potential S_2-radioligands have been investigated. [^{11}C]Ketanserin which has a high affinity but its non-specific binding was too large to allow optimal imaging (2,3). Recently [^{18}F]setoperone has been labelled (4) and tried on baboons (5), but it proved to have a high affinity for the S_2-receptor and a very low non-specific binding but it also had a moderate dopamine affinity. According to Colpaert (6), ritanserin : 6-[2-[4-[Bis (p-fluorophenyl)methylene] piperidino]ethyl]-7-methyl-5H-thiazolo[3,2-a]pyrimidin-5-one, was a pure serotoninergic ligand with a high affinity.

We have undertaken to label this molecule with fluorine-18 (T = 110 minutes) to visualize by PET the serotoninergic receptor in man.

MATERIAL AND METHODS

Fluorine-18 production and resolubilization of $^{18}F^-$

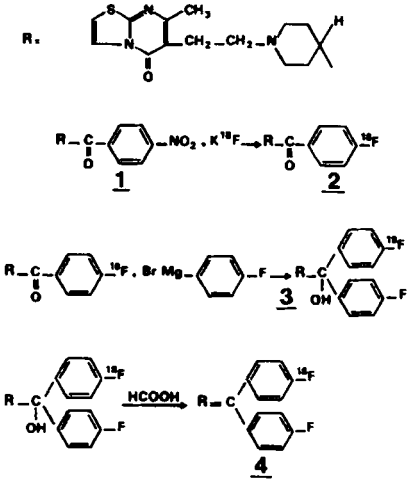
Fluorine-18 has been produced by irradiation of natural water with helium-3, 30 MeV [^{16}O (^{3}He, p) ^{18}F] or enriched water (50 % ^{18}O) with 16 MeV protons [^{18}O (p, n) ^{18}F].

Natural or enriched water was irradiated in a target similar to that described by Kilbourn (7) (Front foil : titanium or Havar foil 12 µm, rear foil : titanium 100 µm, target volume 1 ml). The target was irradiated for one hour with the 520 CGR-MeV cyclotron, with beam intensities ranging from 10 to 20 µA.

Enriched water (50 %) was purchased from the ORIS-CEA Company (ref. ISO 18-2-50). Natural water was obtained from a millipore purification system (Milli Q system, water at 18 megohm x cm).

After irradiation, the water was evaporated at 120°C under a current of nitrogen in the presence of potassium carbonate (2 mg) and kryptofix 2.2.2. (14.5 mg) in a siliconized tube (Vacutainer®, Becton Dickinson, no additive, 5 ml volume).

The solvent, tetramethylene sulfone (sulfolane) used to recover the radioactivity after evaporation of water, was obtained from Aldrich and previously distilled under vacuum on to calcium hydride.



Scheme I : Synthetic route to [^{18}F]Ritanserin

Synthesis and purification

The synthetic method involves three steps (scheme I) :

- a nucleophilic substitution of a NO_2 group by $^{18}F^-$ (4, 8) ;
- addition of the Grignard reagent derived from bromofluorobenzene (Aldrich) to the [^{18}F]fluorophenylketone, and
- a dehydration step of the tertiary alcohol formed in the previous step.

a) Substitution reaction

The nitro-precursor 1 was synthesized in the same way as the nitro-precursor of setoperone (4). Substitution of the nitro group by $^{18}F^-$ took place in a small Pyrex vial fitted with a septum plug in the presence of

potassium carbonate (1.3 mg) the nitro-precursor (5 mg) and sulfolane (500 µl) containing kryptofix 2.2.2. (14.5 mg) at 180°C. After 15 minutes the reaction mixture was diluted with NH_4OH (0.1 N, 15 ml) and passed through a Sep-Pak C_{18} cartridge previously activated by methanol (20 ml) and NH_4OH(0.1 N, 20 ml). The radioactivity was eluted by dichloromethane (10 ml) and the eluate transferred onto a silica Sep-Pak cartridge. The silica was washed with dichloromethane (20 ml) and then eluted with dichloromethane (20 ml) containing 4 % ethanol.

After evaporation of the solvent, the radioactivity was taken up in 800 µl of a CH_2Cl_2 - 3 % B mixture (B = ethanol 94, water 2, ethylamine 4) and was injected onto an HPLC column (Partisil 10, 500 mm x 9 mm, Whatman), solvant CH_2Cl_2 + 3 % B, flow rate 4 ml/min. The radioactivity as well as the U.V. absorption at 254 nm (Waters model 440) of the effluents from the column were monitored. The retention time of product 2 was about 12 minutes. The radioactive peak corresponding to product 2 was collected.

b) Grignard reaction

After evaporation of the solvent, the freshly prepared Grignard reagent (FC_6H_4MgBr) (100 µmol), in dry THF (100 µl) were added. The mixture was heated under reflux for 20 minutes. Then a saturated NH_4Cl solution (1 ml) was added and the product extracted with CH_2Cl_2. This was concentrated and the ^{18}F-product purified by HPLC chromatography as described in paragraph (a).

The retention time of 3 was about 16 minutes. The radioactive peak containing 3 was collected and the solvent evaporated.

c) Dehydration reaction

The product 3 was dissolved in formic acid (1 ml) and heated under reflux for 10 minutes. Then, the acid was evaporated and the ^{18}F-residue was dissolved in CH_2Cl_2 (800 µl). This solution was injected onto the same HPLC column as described in (a). The [^{18}F]Ritanserin was eluted with CH_2Cl_2 + 3 % B. The retention time of ritanserin was 8 minutes. The purity of [^{18}F]Ritanserin has been checked by two thin layer chromatographies :

- kieselgel 60 F_{254} (Merck), CH_2Cl_2 + 5 % Me OH

  Rf Ritanserin = 0.17

- RP-18 F_{254} (Merck), CH_3CN/Water/Phosphate buffer pH 3.5 (35/14/1)
  Rf Ritanserin = 0.67.

The radioactivity of the TLC was detected by a Chromelec 101 detector associated with an analyser multi 8 (Intertechnique).

RESULTS

The yield of the substitution reaction was 50-60 % compared to the ^{18}F radioactivity solubilized with the sulfolane. The percentage of ^{18}F recovered with sulfolane was 85-95 % of the total radioactivity produced. The addition of the Grignard reagent to the ketone gave the alcohol in 40-50 % yield and the dehydration step was quantitative. The total radiochemical yield of the synthesis from ^{18}F was about 20 - 30 % (decay corrected). It is necessary to purify the intermediates 2 and 3 to obtain a good chemical and radiochemical purity of [^{18}F]Ritanserin. All the attempts carried out with only an HPLC purification, at the end of synthesis, did not give a pure product.

After the last HPLC purification, the solvent evaporated and the [^{18}F]Ritanserin was solubilized into an isotonic solution, pH 4, (100 µl CH_3 COOH 10 N, 1 ml NaOH 1 N, 7 ml H_2O) and sterilised by millipore filtration (Millex FG, 0.2 µm). The total time, from the end of the bombardement, to obtain the product ready for injection was 150 minutes.

Starting with 18.5 GBq (500 mCi) of ^{18}F from enriched water, it was possible to obtain 1.1 to 1.5 GBq (30 to 40 mCi) of [^{18}F]Ritanserin with a specific activity of 44 GBq/µmol (1.2 Ci/µmol) at the end of synthesis. The specific activity of [^{18}F]Ritanserin was determined from the last HPLC chromatogram (before injection of the radioactive product a standard curve was made with 20, 30, 40 nmol of cold Ritanserin). The specific activity measured by this method has been checked by an HPLC on a 7 µm Si 60 column (Lichrosorb L = 250 mm, d = 9 mm) (Merck), solvent = chloroform 920, methanol 80, water 5, flow rate = 3 ml/min. The retention time of ritanserin was 7 minutes.

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