NOTE

SYNTHESIS OF N-(^{11}C) METHYL, N-(METHYL-1 PROPYL), (CHLORO-2 PHENYL)-1 ISOQUINOLEINE CARBOXAMIDE-3 (PK 11195) : A NEW LIGAND FOR PERIPHERAL BENZODIAZEPINE RECEPTORS

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SUMMARY

N-(^{11}C) methyl, N-(methyl-1 propyl), (chloro-2 phenyl)-1 isoquinoleine carboxamide-3 (PK 11195) or (^{11}C) MPCI was synthetized in a short time (45 min) and high specific activity (769 Ci/mmol) for the positron emission tomography of peripheral benzodiazepine receptors in heart, kidney and brain. The precursor used was the N-desmethyl MPCI (PCI). The radioactive reagent was the (^{11}C) methyl iodide obtained from nuclear reaction ^{14}N (p,α) ^{11}C via $^{11}CO_2$ and $^{11}CH_3OH$. The chemical purity of the end-product was checked, after HPLC purification, by chemical ionisation mass spectrometry in the parallel synthesis of cold MPCI. The methylation, reversed phase HPLC purification, evaporation times were 10, 6 min respectively so that sterile and isotonic injectable solution of (^{11}C) MPCI ready for use may be obtained, in less than 45 min after the end of nuclear bombardment.

KEY WORDS : ^{11}C, PK 11195, Peripheral, Benzodiazepine, Receptors, Positron, Ligand.

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INTRODUCTION

PK 11195 or N-methyl, N-(methyl-1 propyl), (chloro-2 phenyl)-1 isoquinoleine carboxamide-3 (MPCI) is found to present a high tropism for heart ventricle, kidney, adrenals and to a lesser degree for brain of rat (1). The binding of PK 11195 to target tissues is mediated mainly by peripheral type of benzodiazepine receptors (2). Therefore PK 11195 could be a potent imaging agent in probing physiological roles of these receptors and their relevance in cardiovascular, renal and cerebral human pathologies.

This work deals with the synthesis of (^{11}C) MPCI for positron emission tomography (PET) of peripheral benzodiazepine receptors in view to investigate systematically the capability and limits of the use of this new radiopharmaceutical.

The method of synthesis is based on the N-alkylation of the secondary amide potassium salt (3). In this condition this salt undergoes displacement with great ease and excellent yield (80-90 %) to N-alkylated amide (Fig. 1).

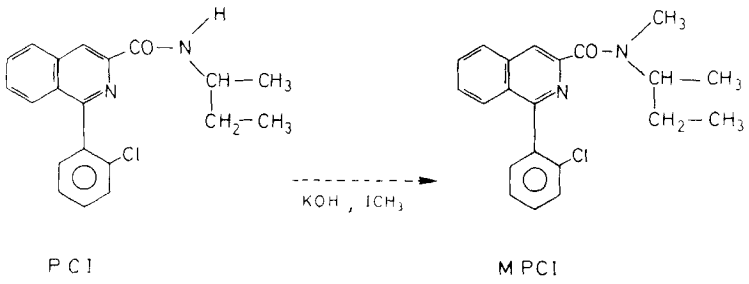

Fig. 1 - Scheme of the synthesis

EXPERIMENTAL AND RESULTS

Analytical system

A Waters 6000A solvent delivery system, U6K injector, 254 nm fixed UV, refractive indice, ionisation chamber detectors and semi-preparative reversed

phase ODS-2 column (Whatman Partisil M9 10/25) were used for purification and quantitation of the labeled compound.

The optimal isocratic separation of compounds of interest were obtained with ethanol-water mixture 75 : 25,v/v as mobile phase for a flow rate of 4 ml/min.

Chemical-ionisation mass spectra (CI-MS) of synthetised product were obtained from JEOL 300D EI-CI magnetic mass spectrometer and JAMA 2000 computer system (JEOL Europe), by direct probe insertion. Methane was used as reactant gas at 1 bar pressure in this CI mode.

The identification of end-product in the parallel synthesis of cold MPCI, was made on the basis of (a) typical presence by methane CI, of quasimolecular ions M+1, M+29, prominents fragments and (b) characteristic isotopic motif (4) easily recognized (5) of 35 Cl and 37 Cl, M+1, : M+1+2 (intensity ratio 3 : 1) from HPLC peak which coeluted with authentic MPCI. The collected fraction of the peak of interest was evaporated (0.2 micromol of end-product) then redissolved in 0.2 ml of ethanol. 0.1 ml of this solution was transferred into the capillary quartz tube and evaporated for the direct probe insertion into the MS.

PCI (I) and MPCI (II)

Pure (I) and (II) were kindly supplied from PHARMUKA France. Analytical characteristics indicated by the manufacturer are Rf (I) = 0.42, Rf (II) = 0.22 in cyclohexane-ethyl acetate 1 : 1, v/v and 0.25 mm Merck silica gel TLC system. HPLC analysis of the spot with normal phase (silica-microporasil 10 microns)/ CH_2Cl_2-B* 99 : 1, v/v system and different reversed phases CN, NH2, C18/CH_2CL2-$CNCH_3$90 : 10, v/v systems, revealed analytically pure products.

(^{11}C) MPCI

Synthetic operations were carried out semi-automatically in a well shielded cell (6). Carbon-11 methyl iodide $^{11}CH_3I$ was transferred (0.3 +/- 0.1 micromol) by a stream of nitrogen at a flow rate of 10 ml/min into the reactional medium

* B = Ethanol-ethylamine-water 96:2:2, v/v

containing 1.2 micromol of PCI previously dissolved in 0.120 ml of DMSO (dimethylsulfoxide) and 20 +/- 5 mg of dry potassium hydroxide. The 3 ml tapered glass tube used for this purpose was tighly closed with a screw cap and silicone septum (Pierce Chem.). It was fitted with 3 needles for gas entrance and solution exit. The reaction occurred instantaneously at room temperature and proceeded regularly upon the arrival of bubling $^{11}CH_3I$ over 6-10 min.

Total crude mixture (0.10 +/- 0.05 ml) was then injected into the chromatograph by gas surpressure, through a teflon tube-glass fiver filter-needle (1 mm diameter) system.

All compounds were baseline resolved in 6 min. DMSO which was poorly detected in the UV trace, exhibit a significant tailing peak in refractive indice trace (Fig. 2) while (^{11}C) MPCI and PCI peaks were perfectly symmetrical.

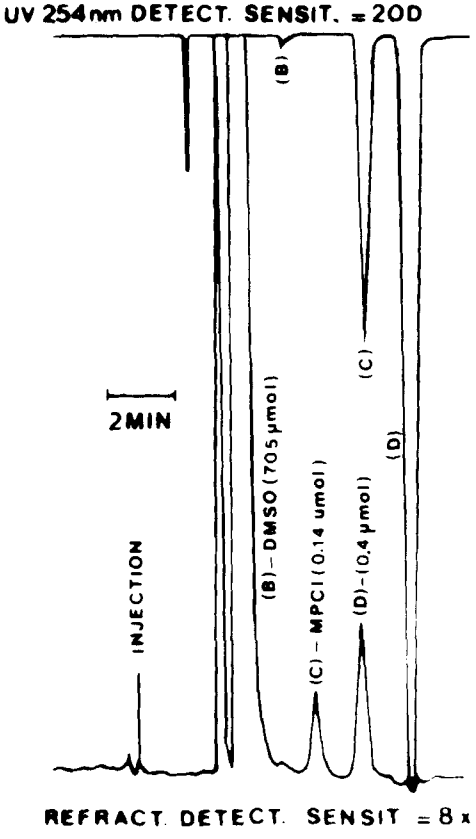

Fig. 2 - Chromatograms of cold MPCI : 254 nm UV (top) and refractive indice (bottom) traces. DMSO (B) poorly seen in UV trace was clearly detected and discriminated from others components. The shift between these two traces is equivalent to 1.3 min.

The early appearance of (^{11}C) MPCI peak (Tr = 4.7 +/- 0.2 min related to that of PCI used in great excess, Tr = 5.8 +/- 0.2 min) fitted nicely with the constraint due to the use of short-lived carbon-11.

Radio-UV chromatograms (Fig. 3) revealed (^{11}C) MPCI chemically and radiochemically pure.

Methane CI mass spectrum of MPCI synthetized with cold CH_3I, displayed a quasi-molecular ions doublet M+1 = 353 (intensity ratio 3 : 1), M+29 = 381 and prominent fragments 297, 281, 225, 207, (Fig. 4) which were identical to those of the reference material.

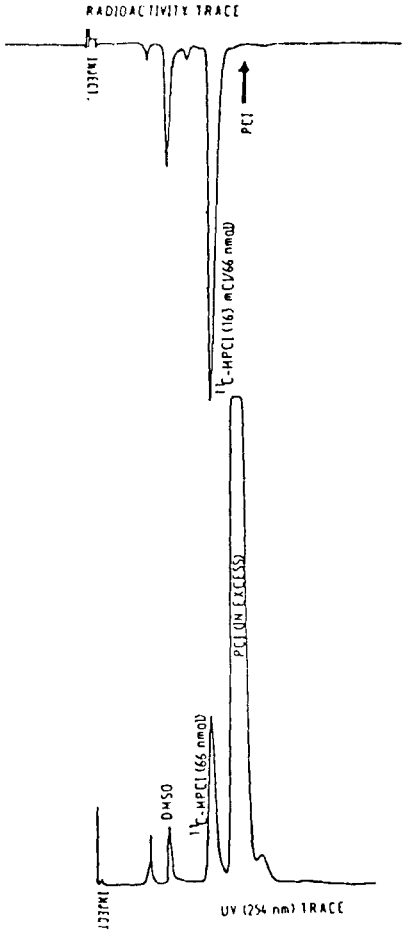

Fig. 3 - Typical HPLC radio-UV chromatograms of (^{11}C) MPCI : separation and purification from the reactional mixture.

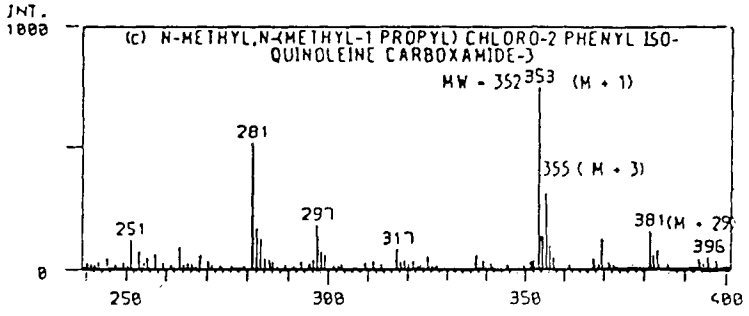

Fig. 4 - The CI mass spectrum (high masses region) using the methane as reactant gas, of HPLC peak which coeluted with the authentic MPCI : ions doublet at m/e 353, 355 and ion M+29 = 381.

The 3 ml fraction of eluant containing the (^{11}C) MPCI was evaporated to 1.5 ml in a 100°C water bath under a nitrogen stream. 3 ml of pH 7 buffered 0.9 % sodium chloride solution were then added. The resulting solution was sterilised by millipore filtration (0.22 micron) into a sterile syringe. Usually 50 % of radioactivity were retained on the filter.

Eight syntheses for the reproductibility testing using 15 min of proton bombardment (30 micro A, 20 Mev) of a nitrogen target (pression = 7 bars, length = 30 cm, diameter = 4.5 cm) gave 78 +/- 18 mCi of (^{11}C) MPCI ready for injection with a mean of specific activity of 769 Ci/mmol.

For kinetic studies of PK 11195 in animals, 30 min bombardment were generally used in order to obtain 70 to 100 mCi after millipore filtration. The preliminary studies using PET with 10 to 25 mCi of ^{11}C-PK 11195 in dogs and monkey confirmed the uptake of this new ligand by the brain and heart.

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