RADIOCHEMICAL SYNTHESIS OF [^{18}F]-FLUOROTHIENYLCYCLOHEXYLPIPERIDINE ([^{18}F]FTCP)

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SUMMARY

The radiochemical synthesis and purification of [^{18}F] N-(1-(2-thienyl)-cyclohexyl)-4-fluoropiperidine [[^{18}F]FTCP] is reported. [^{18}F]FTCP is prepared by [^{18}F] fluoride displacement on a mesylate precursor. The crude products are treated with borane to aid in the removal of an elimination product. Purification of the radiopharmaceutical involves a short silica gel BOND ELUT column and subsequently reverse phase HPLC. The final product has high chemical and radiochemical purity with the radiochemical yield optimized at nearly 30 percent (corrected for decay).

KEY WORDS: [^{18}F]FTCP, N-(1-(2-thienyl)cyclohexyl)-4-fluoropiperidine, phencyclidine, positron emission tomography

INTRODUCTION

Since the discovery of a receptor for phencyclidine, (N-(1-phenyl-cyclohexyl)piperidine (PCP)[1], considerable effort has been devoted to the study of the structure and function of these receptors. Evidence is also being gathered which indicates a correlation between phencyclidine receptor binding and antagonism of methyl-D-aspartate (NMDA) excitatory amino acid receptors.[2] In addition, changes in phencyclidine receptor levels in certain brain regions have been noted in post-mortem studies of Alzheimer's disease.[3]

Although earlier work suggested a common receptor for σ-opiates and PCP,[4] subsequent studies with more selective ligands have demonstrated that these two receptor types are actually distinct.[5]

The regional distribution of phencyclidine receptors in rat brain have been mapped using [^{3}H]TCP (N-(1-(2-thienyl)cyclohexyl)piperidine)

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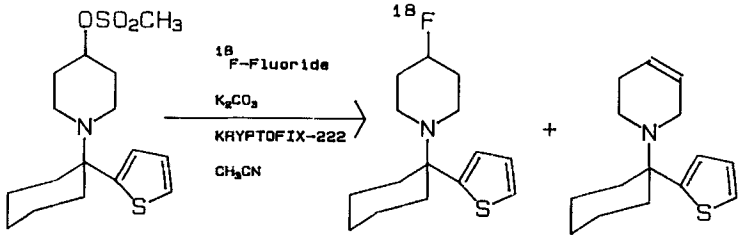
or [^{3}H]PCP combined with in vitro autoradiographic techniques.[6,7,8] The highest density of receptor is found in the hippocampus and cortical area while very low levels are found in the hind brain. Evidence for an endogeneous peptide ligand for the receptor has been presented.[9]

A large number of PCP analogs have been synthesized and evaluated for receptor affinity.[1,10,11,12,13] The thienyl analog, TCP, which displays very high affinity for the phencyclidine receptor, exhibits higher selectivity for the phencyclidine receptor than for the σ-opiate receptor. This selectivity supports the distinction of the two receptor sites.[5]

In an expansion of our interest in studying brain receptor pharmacology using positron emission tomography (PET), we have prepared N-(1-(2-thienyl) cyclohexyl)-4-fluoropiperidine (FTCP).[14] This compound displays suitable in vitro pharmacological properties for development and evaluation of the [^{18}F]fluorine labelled analog as a radiopharmaceutical for PET. This report presents the radiochemical synthesis and purification of [^{18}F]FTCP.

RESULTS AND DISCUSSION

Radiochemistry. The radiochemical synthesis involves fluoride displacement on N-(1-(2-thienyl)-cyclohexyl)-4-methanesulfonyloxypiperidine (TCPOMs), 1, to produce [^{18}F]FTCP (2) and an elimination product, N-(1-(2-thienyl)-cyclohexyl)-3,4-didehydropiperidine (TCPV, 3) (Figure 1). This reaction is analogous to the preparation of authentic FTCP.[14] Initally we evaluated $(CH_3)_4NOH$ as the base to effect the [^{18}F]fluoride displacement. Only relatively low yields of incorporation were obtained. In contrast, the use of Kryptofix-222 (K222) and K_2CO_3[15] provided significantly higher yields (Table 1).



1 2 3

Figure 1

TABLE 1. Effect of base on radiochemical yield.

n[1]	Me_4NOH μmol	K_2CO_3 μmol	K222[2] μmol	TCPOMs mg	YIELD % (corrected)
3	10	-	-	5	11
11	-	11	22	5	34.2 ± 11.5

[1] n is the total number of experiments
[2] K222 is KRYPTOFIX 222

When the reaction temperature was maintained at 90-100 C, a 15 min reaction time was sufficient for completion of the [^{18}F]fluoride incorporation. Radiochemical yields were determined by multiplying the fraction of radioactivity in solution by the fraction of soluble activity which is product. The amount of soluble activity is determined by transfering the reaction solution into a second vessel. Radio-tlc is used to determine the fraction of soluble activity that is product. The yield data calculated in this manner correlated well with the isolated yields.

Glass reaction vessels were satisfactory and only occasional manual agitation was employed during the fluoride incorporation. The incorporation reaction was conducted in 100 μL total volume and employed 2 equivalents of K222, 2 equivalents of K_2CO_3 and 1.3 equivalent of substrate. Ultimately the substrate quantity was reduced from 5 mg to 2 mg, while simultaneously adjusting the other reagents, in order to minimize the problems of purification. The reduced substrate concentration did not affect the isolated yield (Table 2).

TABLE 2. Radiochemical Yield based on activity collected from IP column.

[^{18}F]TCP

n[1]	Conditions[2]	Initial[3] Activity mCi	Product mCi	Time min	SA[4]	Yield corrected (BOS)
11	A	1.88-8.61	0.46-1.42	74-178		34.2 ± 11.5
14	B	2.84-23.1	0.31-5.92	81-103	1302	36.7 ± 8.98
7	C	21.1 -31.3	2.47-5.19	83-90	3691	27.71 ± 7.16

[1] n is total number of experiments
[2] A= 5mg TCPOMS; B= 3mg TCPOMS; C= 2mg TCPOMS; other reagents in same molar ratio as specified in text
[3] Activity delivered from an aliquot of the target water
[4] Average specific activity at beginning of synthesis

Purification: The synthesis of authentic FTCP is accompanied by the production of the elimination side product TCPV.[14] During the purification of the radiolabelled product, this elimination product as well as unreacted starting material must be removed. The high specific activity of the [^{18}F]fluoride causes even very minor side reactions and impurities to become significant. Although the fluoro product and the elimination product could be separated by normal phase HPLC on silica gel, the relatively large amount of elimination product prohibited an efficient separation of the two products from the reaction mixture.

The elimination product can be converted to a very polar diol product by reaction with catalytic osmium tetroxide using N-methyl morpholine N-oxide.[14] This reaction, however, proved too slow for use in the radiochemical synthesis. On the other hand, borane reacts very rapidly with the olefin to produce a polar compound which is easily separated from the fluoro product with a short silica gel column. Following the fluoride incorporation reaction, the acetonitrile solvent was removed under a stream of nitrogen, the reaction vessel was cooled, and 200 μl of borane-THF (1 M) solution was added. This reduction was allowed to proceed for 10 min at room temperature after which it was quenched with 50 μl H_2O. The solvent was removed under a stream of nitrogen and the product was loaded onto a BONDELUTE-SI silica column with CH_2Cl_2. Careful elution with 10% ethyl acetate in hexane allows collection of [^{18}F]FTCP while the olefin-borane product and a significant amount of unreacted mesylate are retained on the column. The effluent is monitored for radioactivity and the radioactivity peak collected. Unfortunately the amount of starting material which remains with the product after this short silica column varies from run to run. Overall the product obtained at this point has very high radiochemical purity. However, an HPLC purification is required in order to achieve high chemical purity.

The chemical components of the radioactive fractions have been evaluated by GCMS (gas chromatography-mass spectrometry) and by HPLC. Neither method indicates the presence of the elimination product. Two

chemical components are apparent in variable concentrations as analyzed by GCMS. One appears to be TCP, the product of borane reduction of the mesylate to the hydrocarbon. Evidence for this component is the mass spectrum and also that one of the HPLC peaks co-elutes with authentic TCP. The identity of the other component is uncertain. This second component exhibits a characteristic base peak of 164 which is consistant with 1-(2-thienyl) cyclohexene. Thermolysis of any of the TCP derivatives may form this compound. Compounds such as FTCP and the olefin TCPV exhibit a base peak of 165. In lower specific activity preparations, the mass peak associated with FTCP may be detected by GCMS.

Prior to HPLC purification, the product, which is obtained from the BONDELUT column in approximately 10 ml, must be concentrated to dryness under a stream of N_2. Great care must be taken after dryness has been reached, because the product is volatile at this point. No loss of product due to volatility is noted until dryness is attained.

HPLC Purification: The fluoro product and the elimination product could be separated by normal phase HPLC on silica gel. However as previously stated, this separation could not be maintained with the relatively large amount of elimination product. Even after removal of the elimination product, other side products were present which precluded the use of normal phase HPLC for the final purification.

The use of a Beckman IP column and a mobile phase of CH_3CN and a buffer was investigated. The first buffer, which consisted of KH_2PO_4 and the sodium salt of octanesulfonic acid (OSA,Na) resulted in broad peaks. The addition of triethylamine to the buffer sharpened the peaks. However, this system failed to separate the fluoro product from the elimination product. Removal of the OSA, Na from the buffer and substituting NaH_2PO_4 for KH_2PO_4 did not adversely affect peak shape. Subsequently, adjustment of the ratio of buffer to CH_3CN provided separation of the fluoro product from elimination product (Table 3). Other bonded phases were investigated, but only the Beckman column was found to effect the required separation. Maintaining the desired separation was mostly a function of column.

TABLE 3: Retention time, α-values, and resolution R of FTCP and elimination product as a function of mobile phase.

CH_3CN %	Aqueous %	Buffer[1] mM	FTCP min	TCPV min	α [2]	R_h [3]
70	30	5	7.89	8.74	1.12	3.55
60	40	5	8.73	9.36	1.09	2.58
50	50	5	10.46	10.94	1.06	1.59
40	60	5	14.02	14.02	1.00	0
70	30	2.5	11.30	12.64	1.14	4.36
65	35	2.5	13.33	14.89	1.14	4.31
60	40	2.5	14.33	15.82	1.12	3.94
70	30	1.7	18.11	20.28	1.13	5.27

[1] buffer strength of each component NaH_2PO_4 and Et_3N. The buffer was adjusted to pH 3 with H_3PO_4
[2] The separation factor.
[3] Resolution at half height.

Application of this chromatography system to the purification of the desired [^{18}F]-labelled product required two analytical columns in series in order to obtain high chemical purity on a routine basis.

Biological: In vitro competitive binding assay of fully decayed ^{18}F-labelled product vs. [^{3}H]TCP was conducted to determine the effective specific activity of the product. The K_i of fully decayed [^{18}F]FTCP was determined utilizing the assumption that the mass of FTCP present was accurately determined by HPLC with UV detection (specific activity 1.9 Ci/μmol). The K_i of the decayed product was 26 nM. During the same assay authentic FTCP exhibited a K_i of 38 nM. Within the variance of the assay, these two K_i's are not significantly different, therefore, the assumption of accurate mass determination by HPLC appears valid.

CONCLUSIONS

We have prepared [^{18}F]FTCP utilizing a 15 min fluoride incorporation reaction followed by a 10 min borane reduction to aid in the removal of the major elimination side product. Subsequently chromatographic methods are used to obtain a radiochemically and chemically homogeneous radiopharmaceutical. The product is obtained with a radiochemical yield of

nearly 30 percent (corrected for decay) in a total synthesis time of approximately 90 min. The radiolabelled product may be formulated for biological experimentation.

EXPERIMENTAL

Authentic FTCP, the elimination product (TCPV), and the mesylate precursor (TCPOMs) were obtained as previously described.[14] [^{3}H]TCP was obtained from the New England Nuclear division of DuPont. KRYPTOFIX-222 was purchased from Bodman Chemical Co. GCMS was performed on a Hewlett-Packard 5970B Mass selective detector interfaced to a 5880A gas chromatograph. Analyses utilized a 12 m x 0.2 mm methyl silicone capillary column. HPLC utilized a Perkin-Elmer Series 4 solvent delivery system, a Kratos Model 783 variable wavelength UV detector, an Ortec NaI(Tl) radiation detection system, and a Beckman Ultrasphere C18-IP column. BONDELUT columns were obtained from Analytichem International.

^{18}F-Fluoride was prepared from a 3 mL $H_2{}^{18}O$ liquid target on the Japanese Steel Works 1710 cyclotron at the National Institutes of Health using the ^{18}O (p,n) ^{18}F nuclear reaction.

Preparation of [^{18}F] N-(1-(2-thienyl)cyclohexyl)-4-fluoro-piperidine [^{18}F]FTCP. A solution of Kryptofix-222 [3.25 mg in 100 μL CH_3CN] and 45 μL of K_2CO_3 (0.097M in H_2O) were placed in a 13 x 100 mm test tube. The tube was placed in a heated block at 90-100 C and the liquid evaporated under a stream of dry nitrogen. To the residue, an aqueous solution of [^{18}F]fluoride, obtained from an $H_2{}^{18}O$ water target after proton bombardment, is added. The water is removed in the 90-100 C hot block under a stream of nitrogen. Three portions of CH_3CN are added and each portion in turn is evaporated to dryness. The procedure requires approximately 10 min for 300 μL aqueous ^{18}F- activity.

A solution of TCP mesylate (2 mg) in 100 μL CH_3CN is added to the dried ^{18}F-activity and heated at 90-100 C for 15 min. After the required time the CH_3CN is evaporated under a stream of nitrogen. The reaction vessel is cooled by immersion in tap water and then 200 μL of 1 M borane-

THF solution is added. After standing at room temperature 10 min, the borane is quenched by addition of 50 μL H_2O. The liquids are evaporated under a stream of nitrogen. The product is loaded onto a 3 mL BONDELUT-SI column, previously rinsed with CH_2Cl_2, with 3 x 500 μL portions of CH_2Cl_2. The column is washed with another 500 μL portion of CH_2Cl_2, and then the product is eluted with 10% EtOAc in hexane. The effluent of the column is monitored with a Cd(Te) radiation detector and the activity collected with arbitrary human selection of limits of the peak. The collected radioactive sample was concentrated to dryness under a stream of nitrogen. The residue was taken up in CH_3CN and loaded onto an HPLC system for final purification. The HPLC system utilized two 4.6 x 250 mm Beckman-IP columns connected in series and an eluant composed of 40% buffer (5mM NaH_2PO_4, 5mM Et_3N, adjusted to pH 3 with H_3PO_4) and 60% CH_3CN. The radioactive product, which eluted at 16 to 18 min, was collected using manual discrimination of start and end of peak. Unreacted TCPOMs, which eluted through the BONDELUT-SI, eluted at approximately 13 min. A small aliquot of this product was removed at this point for subsequent chemical and radiochemical purity evaluation. Starting with 20-30 mCi of aqueous [^{18}F]fluoride, 2.5-5 mCi [^{18}F]FTCP is obtained in a synthesis time of 90 min from EOB.

Prior to formulation the product must be separated from the buffer components. To the remaining sample 500 μL 5% $NaHCO_3$ was added and the solution loaded onto a BONDELUT C-18, which was previously washed with ethanol (2 mL), H_2O (2 mL) and then blown out with air. After loading, the column was dried with air, washed with hexane (1 mL), and then the product eluted with ether. The effluent was monitored with a radioactivity detector and the peak collected. The collected product was concentrated under a stream of nitrogen to remove the ether. Some water remained in the tube. The product could then be formulated as required for subsequent experiments. Typically 10-20 percent of the product activity is lost during this procedure.

Chemical and radiochemical purity. Chemical and radiochemical purity were assayed by HPLC utilizing a single Beckman C18-IP column, the same eluant as the preparative chromatography, and simultaneous mass and

radioactivity detection. The desired product eluted at 8.7 min; unreacted TCPOMs, if present, would elute at 6.5 min. The radiochemical purity was always greater than 95 percent. Chemical purity of greater than 90 percent was attained. The radiochemical purity was also assayed by radio-tlc on silica gel plates. The product had an R_f of 0.5 (50% hexane, 50% ethyl acetate).

Effective specific activity. The effective specific activity was determined in an *in vitro* assay using fully decayed [^{18}F]FTCP as inhibitor versus [^{3}H]TCP. This assay was performed as previously described using a modified tissue preparation.[16] The homogenates were prepared using fresh instead of frozen rat whole brain. The inhibition constant (K_i) for the decayed product was determined to be 26 nM using the Cheng-Prusoff equation, utilizing our calculated K_d for TCP (16.5 nM) from Scatchard analysis.[17]

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