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Radiosynthesis and PET studies of [^{11}C]RJR-2403, a nicotinic agonist

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Summary

(E)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine (RJR-2403, or metanicotine), a nicotinic agonist developed as a cognitive-enhancing drug for Alzheimer's disease, was labeled with carbon-11 using [^{11}C]methyl iodide via a simple and efficient one-step procedure. Regioselectivity of [^{11}C]methylation on the aliphatic nitrogen versus pyridine nitrogen is strongly dependent on the reaction solvent. The reaction in acetonitrile exclusively yields aliphatic N-[^{11}C-methyl]alkylation ([^{11}C]RJR-2403), while only a byproduct is formed when DMF is used as a solvent. Positron emission tomographic (PET) studies in baboon showed a homogeneous distribution of radioactivity within baboon brain with a slow clearance. [^{11}C]RJR-2403 was metabolized very rapidly as evidenced by the fact that at 2 min after intravenous injection only 50% of the total carbon-11 in plasma is parent compound.

Key Words: [^{11}C]RJR-2403; [^{11}C]metanicotine; nAChR; Alzheimer's disease; position emission tomography

Introduction

During the last decade substantial research has been accomplished in the development of PET radiotracers for nicotinic acetylcholine

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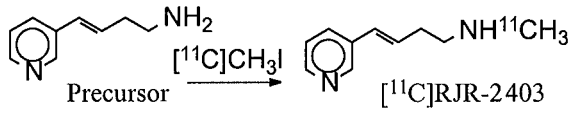

Figure 1. Synthesis of RJR-2403

receptors (nAChR).[1] These receptors play an important role in different physiological states and neurological disorders. High affinity nAChR ligands ($K_i < 50$ pM) such as ^{18}F-labeled epibatidine analogs provided excellent PET images of nAChR in animals.[2,3] A strategy to measure the occupancy of nAChR by nicotine doses equivalent to those when smoking a cigarette became available.[4] Studies of neuronal interactions between the nicotine and dopamine systems have also been explored with this radioligand.[5] However, extremely high toxicity of epibatidine analogues prevented their use as radiotracers in humans.[6] A recent search for specific nAChR tracers of lower toxicity was focused on the development of radiolabeled A-85380 analogs, nicotinic agonists with azetidine 3-pyridyl ether structures. These compounds are less toxic as compared to the analogs of epibatidine, while similar affinity to nAChR is maintained.[7–9]

(E)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine (RJR-2403, or meta-nicotine) is a nicotinic agonist with an affinity of 16 nM to CNS nAChR and three orders of magnitude lower toxicity compared to epibatidine.[6,10–12] This compound has been applied as an analgesic drug with milder side effects compared to opioids and nonsteroidal anti-inflammatory drugs.[13] A potential use of RJR-2403 for the treatment of neurological disorders, such as Alzheimer's disease (AD), was also suggested.[11] In order to better understand the biodistribution, pharmacokinetics and metabolism of this compound *in vivo*, we synthesized [^{11}C]RJR-2403 (Figure 1) by [^{11}C]Ch_3I methylation of the desmethyl precursor and tested it as a potential radiotracer for nAChR. A similar study has been published during the development of this research, although it lacks pharmacokinetics and metabolite data of the drug.[14]

Results and discussion

Chemistry

Regiochemistry of alkylation of compounds containing both aliphatic and aromatic nitrogen is strongly dependent on the reaction conditions.

For example, iodomethylation of nicotine in methanol or acetonitrile gives a mixture of products with predominant *N*-methylation on the pyrrolidinyl ring, while the same reaction in acetic acid leads to exclusive *N*-methylation on the pyridine nitrogen.[15] In the synthesis of 3-(*N*-2-propynyl)aminomethylpyridine, aliphatic *N*-alkylation with 3-bromopropyne was conducted in THF in the presence of triethylamine.[16] In order to achieve selective aliphatic *N*-methylation of (±)-nornicotine with CD_3I, the precursor was initially converted to lithium salt by treatment with BuLi.[17] Alternatively, in the previous synthesis of [^{11}C]RJR-2403 a selective methylation was achieved by using [^{11}C]methyl trifltate as an alkylating agent.[14]

[^{11}C]Methylation with [^{11}C]CH_3I is typically accomplished in high boiling polar aprotic solvents such as DMF[18] or mixtures of DMF and DMSO.[19,20] Although solvents such as ethanol and acetonitrile were also used for reaction, because of the slow reaction rate in these solvents, the yield was usually low (13–15% vs 50–95% in DMF).[18] Having initial failure with the synthesis of [^{11}C]RJR-2403 in DMF using [^{11}C]CH_3I, we studied the possibility to use [^{11}C]formaldehyde in formic acid as an alternative methylation agent. Several examples of reductive methylation of amino groups with [^{11}C]formaldehyde have been reported in literature.[21–23] However, the rate of reaction of the desmethyl precursor with formaldehyde was too slow for the purpose of radiolabeled synthesis. In the presence of a large excess of formaldehyde (290 equivalents) quick formation of RJR-2403 (70% yield in 10–20 min) was observed. Nevertheless, when the quantity of formaldehyde was reduced to 3 equivalents, the yield of RJR-2403 sharply dropped (7% in 30 min). A slow rate of reaction is consistent with a previous observation that reductive methylation by formaldehyde is slower for primary amines compared to secondary amines.[24]

We then carried out an investigation of the solvent effects on this methylation reaction with CH_3I. Although a high excess of CH_3I over the substrate might result in poly-methylation, initial kinetic data (<30 min) provided us with the information regarding relative rates and regiospecificity of the reaction in different solvents. Studies of the reaction showed that the use of acetonitrile as the solvent results in the highest yield of RJR-2403 (34% in 10 min, Table 1). In contrast, no desired product was formed when methylation was carried out in DMF, while most of the precursor was converted to an unknown compound. Addition of triethylamine (TEA, 1.2–2 equivalents) to DMF changed the product composition giving 23% yield of the desired product after 10 min.

Table 1. Methylation of desmethyl RJR-2403[a]

Solvent	TEA	$CH_3I:S$[b]	T(°C)	Yield (%)
Acetonitrile	—	2.3	90	34
DMF	—	2.3	110	0[c]
Methanol	—	10	85	17
Acetonitrile	+[d]	1.7[e]	90	8
DMF	+	1.7[e]	90	23

[a] Reaction in 200 µl of solvent with 1.1 mg (7 µmol) of the desmethyl precursor. The yields after 10 min of reaction were estimated based on the UV peak area of product under HPLC conditions described in experimental section.
[b] $CH_3I:S$ is a ratio of the CH_3I to the substrate.
[c] No formation of RJR-2403 was observed while most of the precursor (85%) was converted to an unknown product ($t_R = 3.7$ min at the analytical HPLC conditions).
[d] Triethylamine (1.2–2 equivalents) present in reaction.
[e] Larger quantity of desmethyl precursor (2.1 mg, 14 µmol) was used.

Table 2. Comparison of the synthesis in Reference 14 with our synthesis

Ref.	Synthesis time (min)	Decay corrected yield (%)	Not decay corrected yield (%)	Specific radioactivity at EOB (Ci/mmol)	Specific radioactivity at EOS (Ci/mmol)	Radio-chemical purity (%)
(4)	28	6–12	2.3–4.5	3460 ± 1050	1310 ± 380	>95
Ours	50	35–42	5.5–6.7	2350–3230	415–570	>98

The results of [^{11}C]methylation in different solvents (Figure 1) paralleled the results of experiments when unlabelled CH_3I. The highest yield of [^{11}C]RJR-2403 was achieved in acetonitrile, while no radiolabeled byproducts were observed in reaction. Decay-corrected yields with respect to [^{11}C]CH_3I for different solvents after 10 min of the reaction (with 0.9 mg of the precursor) were: 50–60% for acetonitrile, 13% for THF and 1% for acetone. When DMF was used as a solvent no formation of [^{11}C]RJR-2403 was observed, while a substantial quantity of radioactivity (37%) was converted to an unknown product (possibly, the result of aromatic *N*-methylation). A comparison of our synthyesis with the one previously reported[14] indicates that despite the fact that our procedure requires more time, it provides a higher yield (Table 2). Our specific activity at the EOB (2790 Ci/mmol) almost equals the specific activity reported previously[14] which is 3460 ± 1050 Ci/mmol ($\pm 30\%$). The difference is within run-to-run variations.

PET studies

PET studies in baboon showed a uniform distribution of radioactivity in most brain regions without the high uptake in nAChR-rich regions such as thalamus that we have observed with F-18 labeled epibatidine and A-85380 analogs.[2,7] A relative lower brain uptake was observed with [^{11}C]RJR-2403 as compared to those tracers as well (total uptake in the brain for F-18 labeled epibatidine, A-85380 analogs and [^{11}C]RJR-2403 consisted of 12–15, 4–5, and 2%, respectively). Pretreatment with nicotine did not produce any significant blocking effect (Figure 2). A

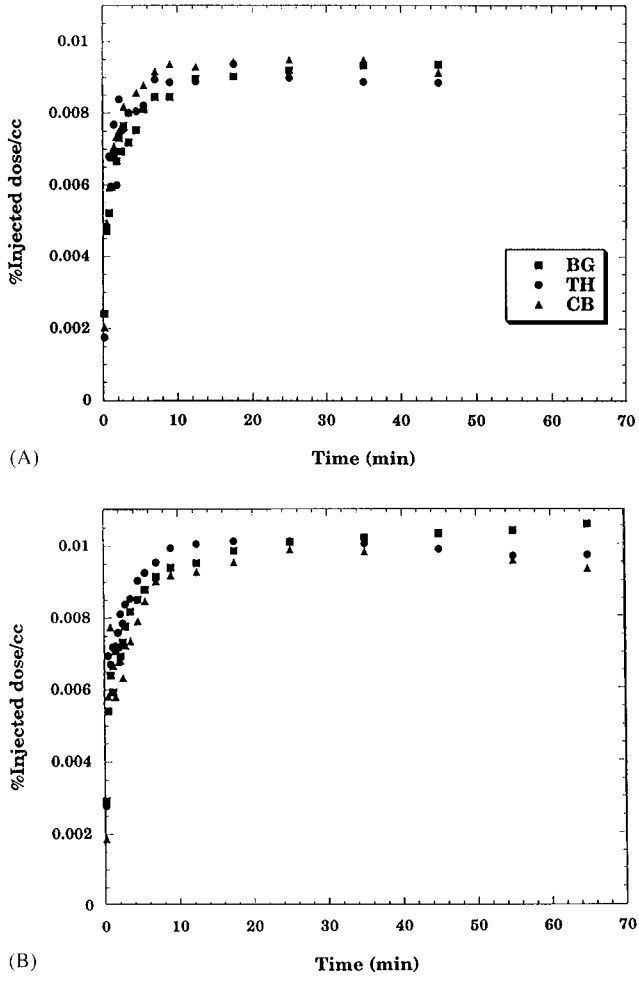

Figure 2. Time activity curves for [^{11}C]RJR-2403 in the thalamus (triangles), basal ganglia (squares) and cerebellum (circles) for studies of (A) baseline (data was processed for 50 min); (B) pretreatment with nicotine

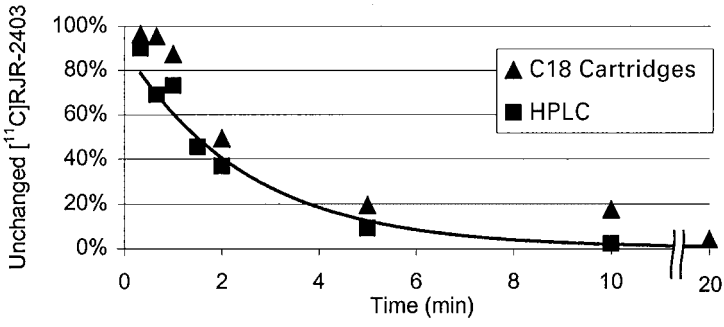

Figure 3. Metabolite essay of baboon plasma. Comparison of data from C18 cartridges and HPLC. The data from HPLC assay are fitted with an exponent $y = 0.90\exp(0.40x)$, where y is unchanged [^{11}C]RJR-2403, and x is the time (min), $R^2 = 0.98$

lack of *in vivo* specific binding for [^{11}C]RJR-2403 is consistent with previously reported observation.[14]

Metabolite assay of baboon plasma indicated an extremely rapid metabolism with only 2–10% of the tracer left unchanged at 10 min postinjection. The amount of the parent compound decreased exponentially (half-life 2 min) with formation of more hydrophilic metabolite(s) as evidenced by earlier elution of radioactivity on the reverse-phase HPLC column. A comparison of metabolite data from HPLC and C18 cartridges showed that the cartridge measurements slightly overestimated the percentage of non-metabolized [^{11}C]RJR-2403 (Figure 3). These results are consistent with previous observations that RJR-2403 is quickly metabolized *in vivo* in human, dog and rat yielding (E)-4-(3-pyridyl)-3-butenoic and 3-pyridylacetic acids along with minor quantities of other metabolites.[25–27]

These studies suggest that [^{11}C]RJR-2403 does not have a sufficiently high affinity to image nAChR *in vivo* with PET. Thus far, A-85380 analogs ($K_i < 50$ pM) currently hold promise as PET tracers to study nAChR in humans.

Experimental

Chemicals were purchased from Aldrich Chemical Co. RJR-2403 ((E)-*N*-methyl-4-(3-pyridinyl)-3butene-1-amine) and desmethyl precursor ((E)-4-(3-pyridinyl)-3-butene-1-amine) were obtained from Targacept

Inc. (Winston-Salem, NC). Methanol was distilled from Mg. Anhydrous acetonitrile and DMF were purchased in Sure-seal® bottles. Purification and analyses of radioactive mixtures were performed with Knauer HPLC pump, in-line UV detector and NaI crystal radioactivity detector. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. The radiochemical purity was determined by radio TLC and analytical radio HPLC in the presence of unlabeled compound as a carrier. HPLC analysis was done using Phenomenex, Shperisorb, ODS-1, 10 μm, 250 × 4.6 mm analytical column; eluent: 75 mM NH_4OAc, pH 4.5–60% CH_3CN; flow rate: 2 ml/min; UV 254 nm; t_R (min): desmethyl precursor, 2.5; RJR-2403, 3.4; impurity formed in DMF, 3.7. For TLC analysis, Macherey-Nagel polygram sil G/UV_{254} plastic-back TLC plates (4 × 8 cm) were used. A short wavelength ultraviolet lamp, NaI well counter and automatic TLC scanner (Berthold Automatic TLC Linear Analyzer) were used as UV and radioactivity detectors. TLC (CH_3CH : water : AcOH 5 : 4 : 3) R_f: impurity formed in DMF, 0.46; desmethyl precursor, 0.60; RJR-2403, 0.53.

Reaction of desmethyl precursor with formaldehyde

Desmethyl precursor (1.5 μl, 11 μmol) was added to the solution of formaldehyde (250 μl, 3.1 mmol of 34% solution in water) in 0.5 ml of formic acid. In the small formaldehyde excess experiment 2.7 μl, 33 μmol of aqueous formaldehyde solution was used, and 250 μl of water was added to bring the volume of the reaction mixture to 0.75 ml. The mixture was heated for 1 h at 80 and 100°C (for small formaldehyde excess experiment), and the samples (3 μl) were periodically taken for HPLC analysis.

Reaction of the desmethyl precursor with methyl iodide (Table 1)

Methyl iodide (1 μl, 16 μmol) was added to the solution of desmethyl precursor (2 μl, 14 μmol) in methanol (0.2 ml). The mixture was heated in a capped vessel for 1 h at 85°C, and the samples (2 μl) were periodically analyzed by HPLC. The reactions in DMF and CH_3CN were accomplished in the same way, except 1 μl (7 μmol) of desmethyl precursor was used in reactions conducted at 110°C (DMF) and 95°C (CH_3CN). For the studies of the influence of TEA on methylation, TEA 6.5 μl (47 μmol) for CH_3CN- and 4 μl (29 μmol) for DMF solvents was

added to solutions containing 2 µl (14 µmol) of desmethyl precursor and 1.5 µl (24 µmol) of CH_3I in 200 µl of solvent, and the reactions were done at 90°C.

Synthesis of [^{11}C]RJR-2403 (Figure 1)

[^{11}C]CH_3I, prepared from [^{11}C] CO_2 using GE PETtrace®, was trapped in solution of desmethyl precursor (0.8–1.5 µl, 6–11 µmol) in 200 µl of solvent (acetonitrile, acetone, DMF and THF). The reaction vessel was heated for 10 min at 90°C for acetonitrile; acetone, 85°C; DMF, 110°C; THF, 90°C. At the end of reaction, 0.5 ml of HPLC eluent was added to the reaction mixture, and the product was separated by semi-preparative HPLC on Waters, µBondpack, 10 µm, 7.8 × 300 mm column using 17 mM NH_4OAc–70% acetonitrile as an eluent at a flow rate of 3 ml/min; $t_R = 15.4$ min. For baboon studies the reaction of the desmethyl precursor (0.9 µl, 6 µmol) with [^{11}C]CH_3I carried out in acetonitrile (200 µl) at 90°C. The fraction collected after HPLC was rotary evaporated. The residue was dissolved in saline (3 ml) and passed through 0.22 µm Millipore filter into a sterile vial. Specific activity was calculated based on the UV peak of RJR-2403 on the semi-preparative HPLC.

PET studies in baboon

Pharmacological profile of [^{11}C]RJR-2403 binding in baboon (*Papio anibus*) brain was determined by carrying out a baseline PET study as previously described.[6] After injection of 2–3 mCi of [^{11}C]RJR-2403, scanning was performed for 70 min with the following scanning sequence: 9 frames of 20 s, 3 frames of 1 min, 2 frames of 2 min, 2 frames of 5 min and 5 frames of 10 min. The brain distribution of [^{11}C]RJR-2403 after pretreatment with nicotine (0.03 mg/kg, intravenously at 5 min prior to radiotracer injection) was compared to that for the baseline study. These two studies were carried out in the same baboon on the same day 2 h apart. Arterial blood was sampled every 2.5 s (OleDich blood sampling machine, Hvidovre, Denmark) for the first 2 min and then at 5, 10, 20, 30, 45 and 70 min. All samples were centrifuged to obtain plasma that was counted for radioactivity, and selected samples were assayed for the presence of unchanged [^{11}C]RJR-2403. Plasma (0.2–0.4 ml) was mixed with 3 ml of water and applied to activated Varian BondElut C18 cartridges (500 mg). A series of four rinses were used to remove the metabolite fractions (2 × 3 ml of water

followed by 2×3 ml of 1 : 1 methanol : water). The radioactivity remaining on the cartridge represented unchanged tracer. The solid-phase analysis was validated by HPLC plasma analysis. Baboon plasma (0.2–0.4 ml) sampled at 0.33, 1, 2, 5 and 10 min was added to 1 ml of acetonitrile, and the mixture was sonicated and centrifuged. Supernatant was analyzed by HPLC with UV detection and radioactivity assay of fractions using the following HPLC conditions: Phenomenex, Spherisorb, 5 μm, 250×10 mm column with 75 mM NH_4OAc, pH 4.5—60% CH_3CN eluent at the flow rate 2 ml/min; t_R 8.5–9.2 min. A sample of unlabeled RJR-2403 was added to the supernatant and four fractions were collected at (1) 0–3 min (void volume); (2) 3–6 min; (3) 6 min until the UV-peak for RJR-2403 appeared; (4) the fraction corresponding to the UV-peak for RJR-2403. The percentage of unchanged [^{11}C]RJR-2403 was calculated based on the radioactivity coeluted with unlabeled standard relative to the total amount of radioactivity injected in the HPLC.

Conclusion

[^{11}C]RJR-2403 ([^{11}C]Metanicotine) was directly synthesized from [^{11}C]CH_3I using a simple one-step procedure. While a high-yield [^{11}C] methylation of the desmethyl precursor (50–60% conversion of [^{11}C]CH_3I into the product) can be accomplished in acetonitrile, only a byproduct is formed when DMF was used as solvent. Baboon biodistribution PET studies revealed homogeneous distribution of [^{11}C]RJR-2403 within the brain. Metabolite analysis showed rapid metabolism of the tracer.

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