PRELIMINARY NOTE

4-[^{125}I] Iodophenyltrimethylammonium Ion, an Iodinated Acetylcholinesterase Inhibitor with Potential as a Myocardial Imaging Agent

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4-[^{125}I]Iodophenyltrimethylammonium acetate (4-I-125 PTMA) was prepared by chloramine-T iodination of N,N-dimethylaniline and subsequent methylation with methyliodide. Purification by thin layer chromatography afforded a product whose specific activity approached the theoretical carrier-free level.

Biodistribution studies in normal ICR mice showed a significant accumulation of 4-I-125 PTMA in the heart tissue, with heart-to-blood ratios of 12.5, 10.4, 7.8, 3.4, 3.4, and 3.3 at 1, 5, 10, 30, 60, and 120 min, respectively. Initial uptake in the heart was greater than 26% of the injected dose per gram. Twenty-five percent of the activity was excreted unchanged by the kidneys during the first 5 min. Less than half of the injected activity was retained in mice at 120 min.

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Wieland and coworkers have synthesized and determined the biodistributions of a series of radiolabeled enzyme inhibitors (*1*) that specifically inhibit the adrenal cortex enzymes, 20 α-hydroxylase, 11 β-hydroxylase and 17 α-hydroxylase. Their finding that some of the inhibitors localized in the adrenal cortex suggests that enzyme inhibitors in general may be useful for the design of radiopharmaceuticals. Acetylcholinesterase (AChE) is an enzyme whose function is to hydrolyze the neurotransmitter acetylcholine to choline and acetate. AChE activity in mammalian tissues is widespread, and, in some species, particularly high levels of activity have been found in the myocardium (*2*). These observations have led us to investigate the possibility of using radioiodinated AChE inhibitors as myocardial imaging agents.

Acetylcholine is a quaternary ammonium salt. Many pharmacologically interesting compounds that possess quaternary ammonium functional groups are generally believed to induce their principal response by altering some normal physiological action of acetylcholine, the substrate of AChE. Simple quaternary compounds, such as the tetraethylammonium ion (*3*), and aromatic quaternary structures, such as phenyltrimethylammonium ion (PTMA, Fig. 1) (*3,4*), are known to inhibit AChE reversibly by blocking the electrostatic attraction between the enzyme and substrate. The fact that phenyltrimethylammonium ion is an AChE inhibitor, coupled with the observation that aryl-iodo compounds are generally resistant to in vivo deiodination (*5*), led us to investigate an iodinated analog of phenyltrimethylammonium ion, 4-iodophenyltrimethylammonium ion (4-I-PTMA, Fig. 1) as a potential myocardial imaging agent. This report describes the synthesis and preliminary biological studies of 4-[^{125}I]iodophenyltrimethylammonium (4-I-125 PTMA).

MATERIALS AND METHODS

Proton magnetic resonance (PMR) spectra were obtained with chemical shifts reported relative to tetramethylsilane, infrared (IR) spectra were recorded, and melting points were determined that are uncorrected. Elemental analyses were performed commercially.* Radioactive iodine was obtained as a carrier-free solution

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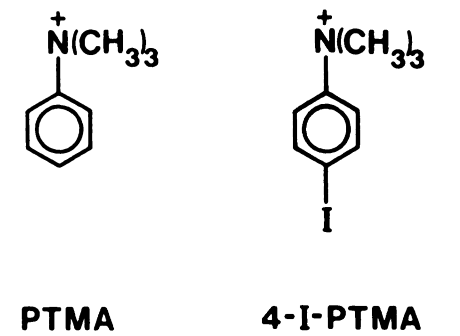

FIG. 1. Structures of phenyltrimethylammonium ion (PTMA) and 4-iodophenyltrimethylammonium ion (4-I-PTMA).

of $Na^{125}I$ in an aqueous solution (reductant free) at pH 8–10 and specific activity of about 350 mCi/ml. Ultraviolet (uv) spectra were also obtained.

Preparation of 4-iodophenyltrimethylammonium iodide (4-I-PTMA). *Method A.* Methyliodide (3.4 g, 24 millimoles) was added dropwise to a solution of 4-iodoaniline (0.5 g, 2.3 millimoles) and sodium acetate (0.38 g, 4.6 millimoles) in anhydrous ethanol (15 ml). The solution was stirred for 84 hr at room temperature. After filtration and washing with diethyl ether, the crude product was recrystallized from hot ethanol/water, yielding 0.77 g (86%) of white crystals mp 194.0 (decomposed).

Method B. Sodium iodide (6.2 g, 41.4 millimoles) in a minimum amount of distilled water was added to a solution of N,N-dimethylaniline (5 g, 41.3 millimoles) in methanol (100 ml). Chloramine-T (9.3 g, 33 millimoles) in a minimum amount of methanol was added dropwise and the solution was gently warmed for 5 min, cooled to room temperature, then evaporated to dryness under reduced pressure. The residue was dissolved in 10% sodium hydroxide and extracted twice with diethyl ether (50 ml). After evaporation of the ether extracts to dryness, the oil that remained was dissolved in anhydrous ethanol (25 ml). Methyliodide (9.1 g, 64.3 millimoles) was added and the solution was heated to gentle reflux for 10 min. Upon cooling to room temperature, a precipitate formed, which was collected by filtration and washed first with cold water then with diethyl ether. Recrystallization from hot ethanol/water yielded 8.6 g (54%) of white crystals, mp 192–193 (decomposed).

Calculated for $C_9H_{13}I_2N$	C: 27.79	H: 3.37	N: 3.60
Found (Method A)	C: 27.76	H: 3.35	N: 3.54
Found (Method B)	C: 27.84	H: 3.39	N: 3.62

Samples of 4-I-PTMA from both methods gave identical infrared and proton magnetic resonance spectra.

IR (nujol mull): 702, 722, 810, 835, 925, 1000, 1120, 1140 cm^{-1} PMR (DMSO-d_6): δ 3.6 (singlet, 9H, $^+N(CH_3)_3$), δ 7.6–8.2 (multiplet, 4H, Ar-H)

UV (water, 4-I-PTMA) λ max 245 nm (ϵ 2630)

UV (water, PTMA) λ max 240 nm (ϵ 2520)

Preparation of 4-[^{125}I]iodophenyltrimethylammonium acetate (4-I-125 PTMA). N,N-dimethylaniline (5 μl) in methanol (100 μl) was mixed with 0.5 mCi of I-125, then 20 μl of a freshly prepared solution of chloramine-T in methanol (1 μg/μl) was added to the mixture to give a total volume of 175 μl. The solution was mixed thoroughly for 5 min before addition of a saturated aqueous solution of sodium metabisulfite (100 μl). The pH of the solution was made basic to litmus by the addition of 10% sodium hydroxide (200 μl), and the solution was extracted twice with diethyl ether (200 μl).

4-[^{125}I]Iodo-N,N-dimethylaniline (4-I-125 DMA) was separated from unreacted N,N-dimethylaniline by thin layer chromatography. The ether extracts were chromatographed on Baker-flex Silica Gel IB-F (7.5 × 2.5 cm) and developed with a methanol/water mixture of 2/1. N,N-dimethylaniline migrated with an R_f of 0.6. 4-I-125 DMA, which migrated to an R_f value of 0.3, was extracted from the silica gel layer with methanol (200 μl), resulting in recovery of about 70% of the activity.

The methanol extract containing 4-I-125 DMA was refluxed for 10 min with methyliodide (50 μl). The reaction mixture was evaporated to dryness. Distilled water (1 ml) containing silver acetate (5 mg) was added to the residue and warmed for 5 min, resulting in the precipitation of silver iodide. After the addition of 0.9% NaCl (2 ml), the resulting suspension was passed through a Millipore filter (0.22 μm, type GS) and diluted with normal saline to an activity of 10 μCi/ml. The radiochemical purity of the product was verified by thin layer chromatography using two chromatographic systems. The R_f values for 4-I-125 PTMA were 0.05 in chloroform/methanol (4/1) and 0.24 in acetone/water (4/1). The uv spectrum of a 1-ml aliquot of 4-I-125 PTMA was recorded for specific-activity determination.

Studies of acetylcholinesterase inhibition. The ability of 4-I-PTMA to inhibit acetylcholinesterase was determined by the method of Fonnum (*6*).

Biodistribution studies. The tissue distribution of 4-I-125 PTMA was determined as a function of time in ICR mice. Normal mice weighing 35–45 g at the time of the study were divided into groups of six and killed under ether anesthesia at intervals of 1, 5, 10, 30, 60, and 120 min following the i.v. administration of 1 μCi of 4-I-125 PTMA in 0.1 ml saline. Samples of blood, atria, ventricles, lungs, kidneys, liver, spleen, stomach, G.I. tract, and muscle were removed and counted in a scintillation well counter. Care was taken to prevent urine from contaminating the carcass when the bladder was removed. The results were expressed as the percentage of injected dose per organ and per gram wet tissue.

Samples of urine were collected after various time intervals and developed on thin layer chromatography using the solvent systems described above. The radiochromatograms were compared with the original sample. The remaining urine samples were evaporated to dryness,

TABLE 1. BIODISTRIBUTION OF 4-I-125 PTMA IN NORMAL ICR MICE. MEAN % DOSE PER ORGAN ± s.d.*

Organ	1 min	5 min	10 min	30 min	60 min	120 min
Blood†	5.26 ± 1.50	4.59 ± 1.26	3.86 ± 0.34	4.19 ± 0.62	3.51 ± 0.89	2.46 ± 0.94
Heart	4.52 ± 0.85	2.99 ± 0.31	1.91 ± 0.27	0.96 ± 0.12	0.66 ± 0.17	0.49 ± 0.15
Atria	0.26 ± 0.09	0.14 ± 0.04	0.092 ± 0.018	0.064 ± 0.024	0.063 ± 0.022	0.050 ± 0.013
Ventricles	4.26 ± 0.77	2.85 ± 0.30	1.82 ± 0.26	0.90 ± 0.13	0.55 ± 0.13	0.44 ± 0.14
Lungs	2.16 ± 0.45	1.37 ± 0.26	0.70 ± 0.09	0.70 ± 0.17	0.49 ± 0.08	0.54 ± 0.20
Kidneys	11.75 ± 5.46	3.04 ± 0.89	2.13 ± 0.59	1.20 ± 0.28	0.84 ± 0.33	0.42 ± 0.09
Liver	7.36 ± 2.11	11.87 ± 1.31	11.42 ± 1.71	6.28 ± 1.05	2.96 ± 0.92	2.09 ± 0.70
Spleen	0.20 ± 0.12	0.28 ± 0.08	0.26 ± 0.04	0.69 ± 0.21	0.51 ± 0.16	0.48 ± 0.11
Stomach	1.02 ± 0.24	1.12 ± 0.11	1.10 ± 0.09	1.05 ± 0.16	0.91 ± 0.13	0.82 ± 0.33
G.I. tract	12.52 ± 1.95	14.03 ± 1.23	13.60 ± 0.89	15.97 ± 2.35	16.76 ± 2.65	13.59 ± 4.17
Carcass	36.88 ± 4.46	34.33 ± 3.81	33.42 ± 5.05	33.02 ± 2.52	25.73 ± 4.55	24.92 ± 4.62
Total	80.8 ± 5.1	73.6 ± 4.3	68.4 ± 5.6	64.1 ± 3.92	57.9 ± 5.5	45.8 ± 9.7

* Six mice studied at each time period.
† Based on 7% of body weight.

redissolved in 0.9% saline, and passed through a Millipore filter (0.22 μm, type GS). The final activity concentration of the filtrate was approximately 1 μCi/ml. A tissue distribution was repeated at 5 min after i.v. administration of 0.1 μCi of this solution. Samples of blood, heart, and lungs were removed and processed as described above.

RESULTS AND DISCUSSION

The para-substituted isomer of I-PTMA can be synthesized easily by method A in an 86% yield. Since this synthesis begins with authentic 4-iodoaniline,† there is no question that the para isomer is obtained by this method. In the PMR spectrum, the aromatic protons appear as an AB pattern (*7*), typical for para-disubstituted benzenes. The coupling constant is approximately 10 cps, as is expected for ortho coupling (*7*).

In a study evaluating the relative binding of a series of substituted phenyltrimethylammonium ions to AChE, Wilson and Quan (*4*) found that nonhydrogen bonding substituents in the ortho position caused decreased binding to the enzyme, whereas the same substituents in the para position increased the binding. For this reason, the para-iodinated analog of phenyltrimethylammonium ion would be expected to have a higher binding strength for the enzyme than the ortho-substituted analog.

Although 4-I-PTMA can be synthesized readily via Method A, this method suffers from the disadvantage of an 84-hr reaction time, which would preclude use of the 13-hr I-123 for imaging studies with this compound. Method B, however, requires only 30 min total reaction time to obtain the desired product in a somewhat lower (56%), but adequate yield. Even though the possibility exists for electrophilic iodination to occur at the ortho position, the NMR spectrum demonstrates that iodination occurs exclusively at the para position.

TABLE 2. BIODISTRIBUTION OF 4-I-125 PTMA IN NORMAL ICR MICE. MEAN % DOSE PER GRAM ± s.d.*

Organ	1 min	5 min	10 min	30 min	60 min	120 min
Blood	2.12 ± 0.60	1.58 ± 0.51	1.36 ± 0.13	1.58 ± 0.16	1.12 ± 0.19	0.89 ± 0.34
Heart	26.43 ± 5.97	16.41 ± 3.01	10.57 ± 1.78	5.35 ± 0.93	3.85 ± 1.03	2.92 ± 1.16
Atria	23.77 ± 4.74	12.08 ± 2.77	8.60 ± 1.67	6.47 ± 0.90	7.29 ± 1.35	6.38 ± 3.72
Ventricles	26.70 ± 6.21	16.72 ± 3.06	10.86 ± 1.75	5.30 ± 0.98	3.33 ± 0.71	2.44 ± 1.06
Lungs	6.74 ± 1.30	4.40 ± 1.16	2.68 ± 0.25	2.28 ± 0.23	1.61 ± 0.29	1.35 ± 0.31
Kidneys	22.87 ± 10.68	5.25 ± 1.78	3.13 ± 0.68	1.90 ± 0.52	1.30 ± 0.52	0.70 ± 0.22
Liver	3.79 ± 1.30	5.85 ± 0.81	5.47 ± 0.76	2.90 ± 0.42	1.28 ± 0.32	1.07 ± 0.45
Spleen	1.48 ± 0.63	2.19 ± 0.59	2.96 ± 0.36	4.37 ± 0.54	2.97 ± 0.36	3.00 ± 1.01
Muscle	1.87 ± 0.48	1.30 ± 0.24	1.37 ± 0.20	1.40 ± 0.29	1.06 ± 0.38	0.96 ± 0.33

* Six mice studied at each time period.

TABLE 3. HEART-TO-BLOOD AND HEART-TO-LUNG RATIOS* FOR 4-I-125 PTMA AS A FUNCTION OF TIME IN MICE

	Time (min)					
	1	5	10	30	60	120
Heart/blood	12.5	10.4	7.8	3.4	3.4	3.3
Heart/lung	3.9	3.7	3.9	2.3	2.4	2.2

* Calculated for mean % dose/g of heart ÷ mean % dose/g of blood or lung.

The 4-I-125 PTMA was prepared by a slight modification of Method B from carrier-free $Na^{125}I$ using a chloramine-T iodination. In order to obtain material with high specific activity, no carrier iodide was added, and the product, I-125-N,N-dimethylaniline, was separated from unreacted N,N-dimethylaniline by chromatography on silica gel eluted with mixture of methanol/water (2/1). Using this system, the product and starting material are easily separated, with 4-I-125-N,N-dimethylaniline and N,N-dimethylaniline migrating with R_fs of 0.3 and 0.6, respectively. The 4-I-125 PTMA was recovered from the silica-gel strip in about 70% yield by cutting out the portion of the strip containing the desired material followed by extraction into methanol. Quaternization was accomplished by refluxing the methanol extract with methyl iodide for 10 min, which gave an 80% radiochemical yield for this step. After evaporation to dryness and dissolution in distilled water 4-I-125 PTMA was converted to its acetate salt (to increase water solubility) by treatment with silver acetate followed by dilution with normal saline. Millipore filtration was used to sterilize as well as to remove precipitated silver chloride and silver iodide. The overall radiochemical yield, based on $Na^{125}I$, was approximately 45%, making this synthesis suitable for use with I-123.

There was no detectable uv absorbance for a 1-ml sample of 4-I-125 PTMA containing 10 μCi activity at either 240 nm or 245 nm; therefore, the maximum possible concentrations of 4-I-PTMA and PTMA were $3.8 \times 10^{-7} M$ and $4.0 \times 10^{-7} M$, respectively. Based on an activity concentration of 10 μCi/ml, this corresponds to a specific activity of at least 26 Ci/millimole. Since the unreacted starting material (N,N-dimethylaniline) should have been removed by chromatography, the specific activity of the product should approach the theoretical maximum of about 2,200 Ci/millimole.

The biodistribution of 4-I-125 PTMA was determined in mice at intervals of 1, 5, 10, 30, 60, and 120 min after i.v. injection, the results of which are shown in Tables 1, 2, and 3. The compound clears very rapidly from the blood, with only 4.6% of the injected dose remaining in the circulation at 5 min after injection. Uptake in the heart is rapid, with 26.7 and 23.8% dose/g in the ventricles and atria, respectively, at 1 min after injection. Although the activity in the heart does not remain constant, clearance is slow enough so that heart-to-blood ratios of greater than three are maintained for all times studied.

Excretion of 4-I-125 PTMA is primarily through the kidneys. Greater than 50% of the injected dose was excreted in the urine after 120 min. Chromatography of urine samples after various time intervals indicate that the compound is excreted unchanged, with no evidence of deiodination or demethylation (Table 4). After evaporating the urine samples to dryness and redissolving the excreted material in saline, we repeated the biodistributions, looking at samples of heart, blood, and lungs at 5 min after injection. These results are shown in Table 5. In comparison with the distribution of the original sample, it is clear that there is no substantial difference between the two biodistributions, again indicating that excretion occurs with little or no metabolism.

The high heart-to-blood and heart-to-lung ratios obtained in mice with 4-I-125 PTMA suggest that 4-I-123 PTMA may be useful for imaging the heart in hu-

TABLE 4. CHROMATOGRAPHY OF 4-I-125 PTMA ON SILICA GEL

Solvent system:	$CHCl_3$/MeOH (4/1)		Acetone/H_2O (2/1)	
Compound:	I-125 PTMA	I-125 NNDMA	I-125 PTMA	$^{125}I^-$
R_f:	0.05	0.9	0.24	1.0
Injected sample	>99%	<1%	>99%	<1%
Excreted sample	>99%	<1%	>99%	<1%

TABLE 5. BIODISTRIBUTION OF EXCRETED 4-I-125 PTMA IN NORMAL ICR MICE AT 5 MIN AFTER ADMINISTRATION

	% Dose/organ*	% Dose/g*
Blood	3.73 (3.91–3.57)	1.53 (1.56–1.47)
Heart	2.81 (2.96–2.71)	19.40 (24.67–15.06)
Lungs	1.28 (1.31–1.24)	3.54 (3.82–3.36)

* Mean and (range); three mice per test.

mans. Although in vitro studies demonstrated that 4-I-PTMA inhibits acetylcholinesterase at concentrations as low as $10^{-5}M$, we have no evidence that the heart uptake is related to binding to acetylcholinesterase or acetylcholine receptors. In vitro studies are currently in progress to determine whether the binding to heart muscle is due to specific interaction of 4-I-125 PTMA with acetylcholinesterase, and the results will be reported later. The apparent in vivo stability, the simple, rapid synthesis, the high heart activity, and the high heart-to-blood and heart-to-lung ratios, indicate that 4-I-125 PTMA warrants further study as a potential myocardial imaging agent.

FOOTNOTES

* Robertson Microanalytical Laboratory, Florham Park, NJ.
† Aldrich Chemical Company.

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